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A STUDY OF INTERMOLECULAR COMPLEXES OF BIS (p-CHLOROPHENYL) ACETIC ACID AND SOME BIOLOGICALLY SIGNIFICANT COMPOUNDS

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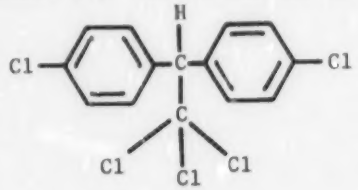
The mode of action of chlorinated hydrocarbons has received much recent attention, and several elegant studies have attempted to relate binding and complexation phenomena with toxic effects.[1-7] The occurrence and persistence of the compound 1,1,1-trichloro-2,2-bis (p-chlorophenyl) ethane, p,p'-DDT (I),in the environment, in addition



(I)

to the fact that its biochemical mode of action has not been unequivocally established, has resulted in a continued effort to determine its molecular interaction capabilities. To date, the study of complexation of p,p'-DDT with biological compounds has been limited due to its insolubility in aqueous systems. However, attempts have been made to establish, on a molecular level, the behavior of p,p'-DDT complexes in nonaqueous media, employing high resolution nuclear magnetic resonance (NMR) spectroscopy.[5,8,9] The use of the NMR technique to study the behavior of such complexes permits more precise characterization of the possible molecular associations involved.

An important metabolite of p,p'-DDT is bis (p-chlorophenyl) acetic acid (p,p'-DDA), II. Since the specificity of biologically

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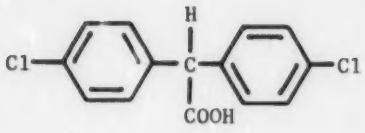

(II)

active materials lies in the structure of the complexes they form with macromolecular receptors[10], any qualitative data on the chemistry and function of agent-receptor complexes yields insight into the pharmacodynamic behavior of the agent molecule. Various chemical and physical techniques including spectrophotometry, microcalorimetry, chemical kinetics, structure-activity correlations involving chemical modifications of either the agent or the receptor, and electrophoresis have been used to establish specific molecular interactions of biochemical importance. A significant limitation of these methods is their inability to reflect relevant structural detail inasmuch as they measure large alterations in chemical and physical properties. Suitable models for this study are the complexes that p,p'-DDA may form with both bovine serum albumin (BSA) and human serum albumin (HSA). The vascular transport of p,p'-DDA may be an important step in the excretion of p,p'-DDT from the liver, the principal metabolic site.[11] The solubility of p,p'-DDA in slightly alkaline aqueous solutions and the ease of interpreting the NMR spectrum make this compound unique for the study of complexation with biological compounds. This study was made to directly test the existence of binding of p,p'-DDA with BSA and HSA and to examine the structure of the complexes.

EXPERIMENTAL

Proton NMR spectra were obtained on a Varian Associates HA-100 high resolution NMR spectrometer operating at 100 MHz. Chemical shifts were measured by a Hewlett Packard Model 5512A electronic frequency counter which displays the pen position in Hz from the lock signal, in this case an external capillary of tetramethylsilane. Temperature control and measurements were made with a Varian Associates V-4333 variable temperature probe and a V-4343 temperature controller.

All solutions for NMR studies were prepared with D_2O as solvent and p,p'-DDA concentrations are expressed in moles/liter (M). BSA and HSA concentrations are in percent w/v i.e., in grams per 100 ml final volume.

Except for the variable temperature studies, the NMR measurements were obtained at 31.5 ±1.0°C. The spin-spin relaxation times,

T_2, were obtained from a measurement of line width.[12] Each reported value of T_2 represents the mean of at least 6 measurements. All pD adjustments were made with DCl-D_2O and NaOD-D_2O solutions. pD measurements were made with a Beckman Expandomatic pH meter and are uncorrected for deuterium isotope effects.

Commercial sources used were as follows: D_2O, tetramethylsilane, and NaOD, Wilmad Chemical Co., Buena, N. J.; p,p'-DDA, Aldrich Chemical Co., Milwaukee, Wis.; crystallized bovine serum albumin and human serum albumin, Sigma Chemical Co., St. Louis, Mo.

RESULTS AND DISCUSSION

The NMR spectrum of 0.25 M solution of p,p'-DDA is shown in Figure 1. The aromatic protons are easily identified by their characteristic low field position at 7.54 ppm. The methine proton on the α carbon atom gives rise to a signal at 5.34 ppm. The remaining peak at 5.23 ppm is due to a water resonance arising from HDO generated by the replaceable hydrogen atoms of the p,p'-DDA, BSA, and HSA molecules.

To study the effects of BSA and HSA on the p,p'-DDA molecule, 0.25 and 0.40 M solutions of p,p'-DDA containing 0, 1.25, 2.50 and 4.00% of BSA and HSA respectively were examined. Figure 2 shows the results of a typical series of measurements. The relaxation rates of the aromatic protons are changed by a larger factor (x5) than the relaxation rate of the α-methine proton (x2). Thus the binding phenomenon may be interpreted in terms of preferential stabilization of the aromatic rings by binding sites on the BSA and HSA molecules.

Since the observed broadening may be nonspecific and may be due to alternate relaxation mechanisms such as intermolecular interactions between p,p'-DDA molecules, control experiments were performed on a series of solutions at varying concentrations of p,p'-DDA. Chemical shifts decreased as the concentration of p,p'-DDA increased, with a maximum shift of 18.2 Hz upfield for the aromatic resonances between 0.05 M and 2.00 M solutions. Differences in relaxation rates comparable to those observed on addition of BSA, however, were not observed.

The large upfield shift of the aromatic proton resonances may be attributable to extensive association of p,p'-DDA molecules at higher concentrations, resulting in an increase of shielding effects above and below the aromatic rings.[13] Further, an apparent collapse of the AA'BB' multiplet[14] of the two sets of aromatic protons to a single resonance line occurred at concentrations exceeding 0.20 M p,p'-DDA, under our conditions. Thus, specific molecular interactions involving solute or solvent molecules preferentially influence either the AA' or the BB' protons of the aromatic rings of

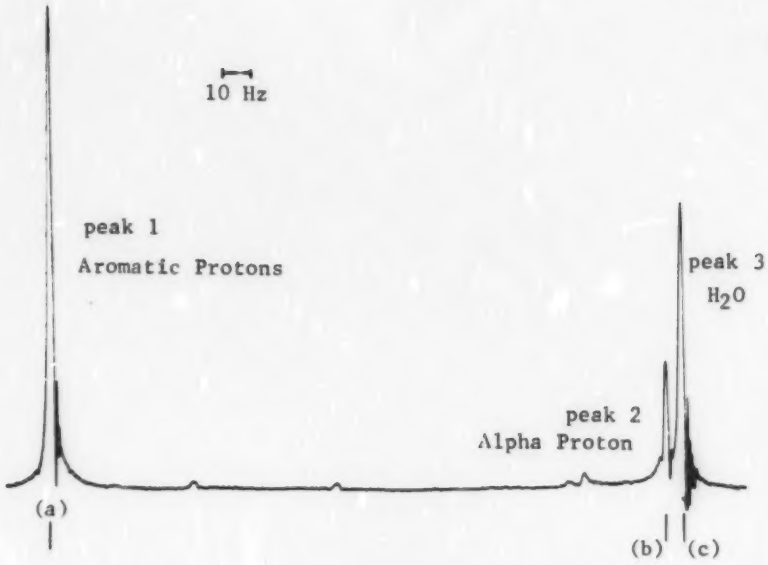

Figure 1

The NMR spectrum of 0.25 M solution of p,p'-DDA
(a) Aromatic protons (753.7 Hz)
(b) Benzylic proton (533.7 Hz)
(c) Water protons (523.4 Hz)

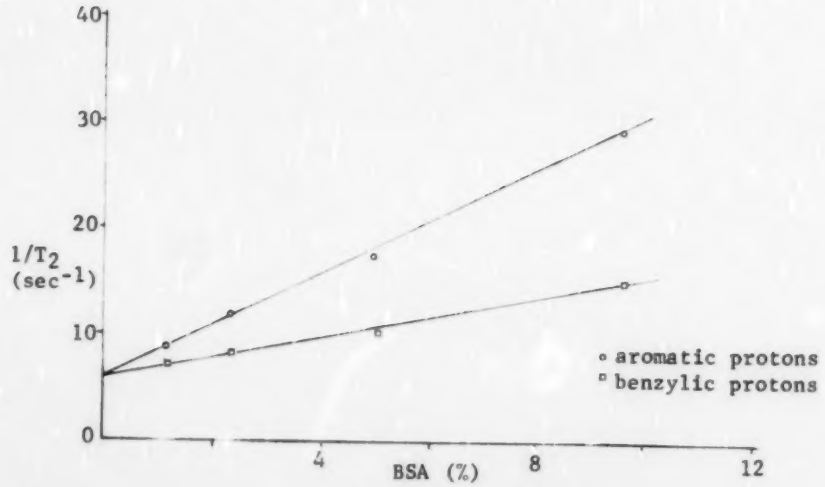

Figure 2

Plots of $1/T_2$ of a 0.25 M solution of p,p'-DDA vs BSA concentration.

Table 1. Effects of Ionic Strength on Chemical Shifts (Hz) and $1/T_2$ (Sec^{-1}) Values of p,p'-DDA Spectral Lines

	Aromatic Protons			Tertiary Protons		
NaCl Conc. M	Chemical Shift (Hz)	$1/T_2$	(Sec^{-1})	Chemical Shift (Hz)	$1/T_2$	(Sec^{-1})
A. 0.25 M p,p'-DDA, 5% Albumin						
0.0	761.2	9.97	(0.18)	531.7	8.82	(0.53)
0.1	760.5	16.70	(0.42)	532.5	14.85	(0.16)
0.3	760.8	21.62	(0.22)	532.1	17.61	(0.09)
0.7	759.9	27.15	(0.27)	532.0	19.18	(0.23)
1.0	759.3	31.11	(0.5)	531.7	20.75	(0.31)
2.0	757.3	64.05	(0.31)	533.7	37.81	(0.11)
B. 0.25 M p,p'-DDA						
0.0	763.5	5.28	(0.62)	533.2	4.99	(0.10)
0.1	762.4	5.49	(0.35)	532.6	5.37	(0.35)
0.3	761.1	5.31	(0.49)	532.7	5.59	(0.26)
0.7	760.4	5.21	(0.3)	532.4	5.65	(0.08)
1.0	760.0	5.87	(0.22)	533.8	5.87	(0.32)
2.0	759.8	6.15	(0.48)	532.8	6.09	(0.46)

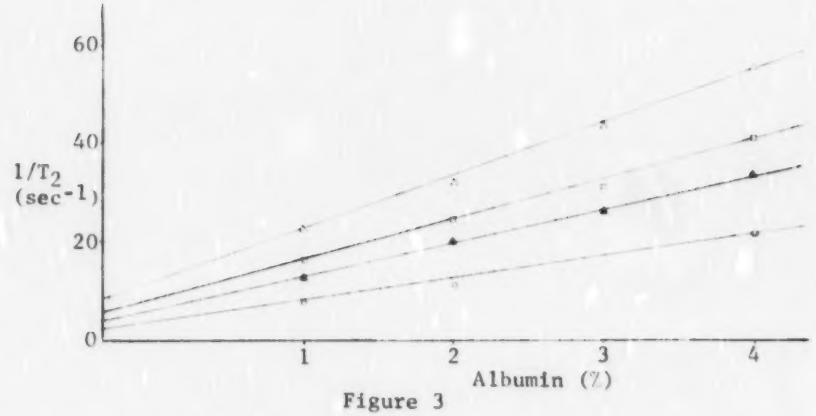

Figure 3

The effect of pD on the relaxation rates of 0.25 M solutions of p,p'-DDA at varying albumin concentrations (%). Δ p,p'-DDA·BSA pD 7.45; □ p,p'-DDA·BSA pD 8.25; o p,p'-DDA·BSA pD 9.70; ▲ p,p'-DDA·HSA pD 7.70.

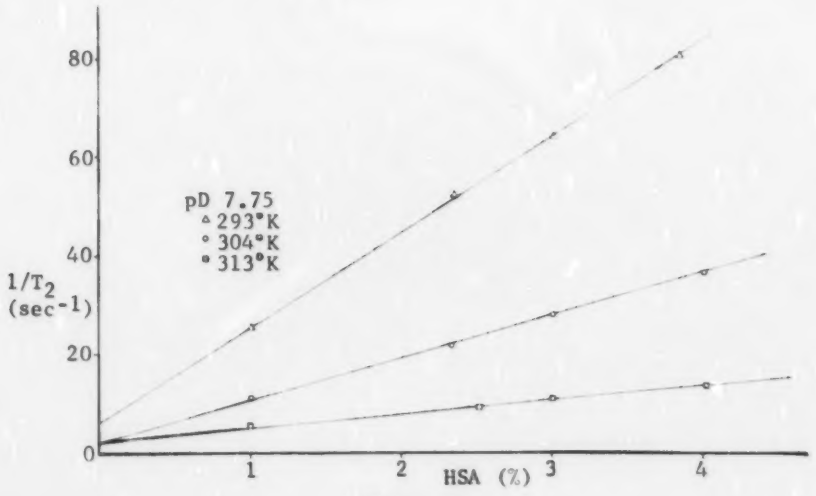

Figure 4

Temperature dependence of relaxation time vs HSA concentration.

p,p'-DDA at higher concentrations.[15,16] This effect facilitated relaxation measurements of the aromatic ring protons.

Control experiments in which the chemical shifts of the AA' protons and the BB' protons were measured as a function of increasing BSA concentration have shown that virtually no contribution to the broadening of the aromatic proton resonances can be attributed to small internal chemical shift variations and incipient resolution of the complex, collapsed AA'BB' multiplet.

To study the effects of pD on the p,p'-DDA interactions with BSA and HSA, solutions of 0.25 M p,p'-DDA containing 0, 1.0, 2.5, 3.0, and 5.0% BSA and HSA were examined over the pD range 7.45-9.75. The measured relaxation rates were quite dependent on pD, as expected from a specific binding mechanism (Figure 3); the largest values are at pD 7.45 and decrease as the pD is increased, a finding consistent with the known behavior of albumin binding phenomena. BSA is known not to bind above pD 10.5 because of protein denaturation.[17]

The effect of ionic strength on the p,p'-DDA albumin interaction was studied with a solution of 0.25 M p,p'-DDA plus 5% BSA, together with a control solution of 0.25 M p,p'-DDA, in the presence of varying amounts of 0.0-2.0 M NaCl. The results of the line width measurements are given in Table 1. The increase in line width with increasing ionic strength is pronounced for p,p'-DDA·BSA but small for p,p'-DDA alone; the chemical shifts of the peaks of both solutions change in the same direction, upfield with increasing NaCl concentration.

These line width measurements are useful in deciding on a mechanism to explain p,p'-DDA·BSA binding. Since the energy of electrostatic attractions varies inversely with dielectric constant,[18] one would expect an ionic bond between p,p'-DDA and BSA to be weakened by addition of salt. The reverse of this situation is observed. These findings suggest that p,p'-DDA·BSA interaction is hydrophobic, as expected, since the aromatic rings are the primary site of binding as discussed above.

The temperature dependence of the NMR spectra of p,p'-DDA with HSA is illustrated in Figure 4. Line widths for both p,p'-DDA·BSA and p,p'-DDA·HSA complexes were found to increase as the temperature decreased. This effect is probably due to an increase in the fraction of p,p'-DDA bound as the temperature decreases, since changes in line widths for unbound p,p'-DDA are not sufficient to account for the marked increase in relaxation rates.

To test the specificity of the binding sites on the albumin molecules, a series of inhibition studies was made. In these experiments an attempt was made to displace the p,p'-DDA from the

Table 2. Inhibition Studies with p-Chlorophenylacetic Acid; $1/T_2$ Values (Sec^{-1})

Sample Description	p,p'-DDA Spectral Lines				p-CPA Spectral Lines			
	Aromatic Protons		-CH-		Aromatic Protons		-CH-	
0.25 M p,p'-DDA	3.11	(0.16)	2.97	(0.31)	----------		--------	
1.20 M p-CPA	----------		----------		3.45	(0.19)	3.19	(0.25)
0.25 M p,p'-DDA 1.20 M p-CPA	OVERLAP		3.79	(0.42)	OVERLAP		3.57	(0.11
0.25 M p,p'-DDA 5% BSA	----------		----------		10.24	(0.48)	5.33	(0.35)
0.25 M p,p'-DDA 1.20 M p-CPA 5% BSA	8.86[a]	(0.45)	4.97	(0.22)	9.18[a]	(0.58)	4.45	(0.18)

[a]Slight overlapping of peaks occurred; these values are estimates.

albumin by adding a large excess of another chemically analogous compound, p-chlorophenyl acetic acid (p-CPA).

In the NMR spectrum of p-CPA the methylene resonances are displaced upfield to 390.5 Hz relative to the tertiary proton in p,p'-DDA. Thus the relaxation rates of both these peaks in a mixture of these two compounds can be measured. Unfortunately, the aromatic proton resonances overlap somewhat and measurements of the relaxation rates of these peaks, where given, represent estimates. Table 2 gives the line widths of the resonance peaks of interest. The control experiments indicate that both p,p'-DDA and p-CPA bind to BSA. Furthermore, the spectrum of the p,p'-DDA, p-CPA and BSA mixture shows slightly less broadening of the p-CPA peaks and much less broadening of the p,p'-DDA peaks compared with controls. This difference may be due to a competition between p-CPA and p,p'-DDA for the binding sites on the serum albumin molecule.

SUMMARY

The results of this investigation have shown that with the use of high resolution NMR relaxation measurement studies, some insight into the importance of molecular binding of p,p'-DDA with BSA and HSA may be obtained. The binding phenomena may be interpreted in terms of preferential stabilization of the aromatic rings by binding sites on the BSA and HSA molecules. This was demonstrated by the greater relaxation rates found for the aromatic protons of the p,p'-DDA than those of the α-methine proton. These relaxation rates were quite pD dependent as would be expected from a specific binding mechanism. The binding of p,p'-DDA·BSA and p,p'-DDA·HSA is hydrophobic in nature since a direct relationship was found with ionic strength and relaxation times. The relaxation rates of both complexes increase with decrease in temperature.

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Synthesis of Radioactive Iodine^{131} Analog of DDT[1]

By Jens A. Jensen and George W. Pearce

The iodine^{131} analog, 1,1,1-trichloro-2,2-bis-(*p*-iodo^{131}-phenyl)-ethane, of DDT has been prepared by the steps

$$C_6H_5NH_2 + HNO_2 \xrightarrow[NaNO_2]{HCl} C_6H_5N_2Cl \quad (1)$$

$$C_6H_5N_2Cl + NaI^{131} \longrightarrow C_6H_5I^{131} \quad (2)$$

$$2C_6H_5I^{131} + CCl_3CHO \xrightarrow{ClSO_3H} \begin{matrix} I^{131}C_6H_4 \\ I^{131}C_6H_4 \end{matrix}\!\!>\!CHCCl_3 \quad (3)$$

The method of Lucas and Kennedy[2] was used for the first two steps. Twenty-two millimoles of aniline was converted to 15 millimoles of iodobenzene, using 22 millimoles of NaI in which 25 millicuries of NaI^{131} was incorporated. The steam distilled product plus 6 millimoles of inactive iodobenzene was condensed with 8 millimoles of chloral using chlorosulfonic acid as condensing agent. Recrystallization of the crude product from 1 to 1 acetone–alcohol gave 1.42 g. (2.6 millimoles) of DI*DT, m.p. 173–174°, 25% yield based on iodobenzene. Activity recovery was 22.4% corrected for decay, but not for inactive iodobenzene added. Specific activity was 2.5 microcuries per milligram. The synthesis can be completed in 2–3 days.[3]

(1) From the Technical Development Branch, Communicable Disease Center, U. S. Public Health Service, Federal Security Agency, Savannah, Georgia.

(2) "Organic Syntheses," Coll. Vol. II, John Wiley and Sons, Inc., New York, N. Y., 1946, p. 351.

(3) For detailed description order Document 3488 from American Documentation Institute, 1719 N Street, N. W., Washington 6, D. C., remitting $1.00 for microfilm (images 1 inch high on standard 35-mm. motion picture film) or $1.20 for photocopies (6 × 8 inches) readable without optical aid.

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Synthesis of DDT Labeled with Carbon-14 in the Tertiary Position

George W. Pearce and Jens A. Jensen

Communicable Disease Center, Public Health Service, U. S. Department of Health, Education, and Welfare, Savannah, Georgia

A method has been developed for the synthesis of radioactive Dichlorodiphenyltrichloroethane (DDT) in good yield in which the tertiary carbon is labeled.

Fields *et al.* (*1*) have developed a satisfactory procedure for incorporating C^{14} in the benzene ring. This procedure presumably was employed in a previously reported labeling of DDT with C^{14} (*2*). However, the complete synthesis of DDT by this procedure would involve at least seven major steps, with the consequent tendency towards low over-all yields. In the authors' procedure, only four major steps are necessary. Schematically, they are as follows:

$$BaC^*O_3 \longrightarrow CH_3C^*OOEt \xrightarrow{LiAlH_4} CH_3C^*H_2OH$$

$$Cl_2 \rightarrow CCl_3C^*HO \longrightarrow DDT$$

The synthesis of carboxy-labeled ethyl acetate was carried out by special adaptations of the methods of Sakami *et al.* (*3*), Van Bruggen *et al.* (*4*), and Ropp (*5*). The ester was reduced to ethyl alcohol by a modification of the method of Cox and Turner (*6*). Chlorination was carried out in a specially designed apparatus, and the subsequent condensation of chloral with chlorobenzene followed the work of Mosher *et al.* (*7*).

Starting with 50 mM of barium carbonate containing 20 mc of activity, 15 g of crude DDT (42% yield based on EtOH) was obtained. Two crystallizations from ethyl alcohol yielded 6.11 g of *p,p'* DDT (17% yield) having a melting point of 107–107.5° C. The specific activity of the product was approximately ½ mc/g.

The residue is currently being treated to recover additional amounts of the *p,p'*-isomer as well as the *o,p*-isomer.

It is planned to submit a detailed report of this work elsewhere.

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TRACER STUDIES—INSECTICIDES

Preparation of Carbon-14 Labeled DDT

GEORGE W. PEARCE and JENS A. JENSEN

**Communicable Disease Center, Public Health Service,
U. S. Department of Health, Education, and Welfare, Savannah, Ga.**

RADIOACTIVE DDT LABELED WITH CARBON-14 has been used in studies of the disposition of the compound in insects (*2*, *4*, *5*). The writers have been advised that the labeled material used contained carbon-14 in the benzene ring. Presumably the ring was labeled through the procedure described by Fields *et al.* (*3*). DDT labeled by this method is necessarily costly, owing to the low over-all yield inherent in any procedure involving a mutiple of synthesis steps. In the case of studies involving insects, only milligram quantities of the labeled compound are needed as a rule. For toxicological studies with higher animals, relatively much greater quantities are required. As labeled DDT was desired principally for the latter purpose, study of a more efficient synthesis was undertaken.

Methods have been developed for preparing $CH_3C^{14}H_5OH$ in good yield (*1*). Chlorination of ethyl alcohol to chloral, and subsequent condensation with chlorobenzene to form DDT, appeared to be most direct, promising relatively high over-all yield. It was apparent from the literature and from preliminary trial runs that the chlorination of the alcohol was highly complex and would be the limiting reaction as far as yield of DDT is concerned. Consequently, considerable time was devoted to developing a satisfactory chlorination procedure.

An outline of the synthesis steps employed and the yield obtained has been published (*7*).

Ethyl Acetate ($CH_3C^{14}OOC_2H_5$) Barium carbonate (1.20 grams) containing 20 millicuries of activity was added to 8.65 grams of inactive barium carbonate (total 50 millimoles) and the mixture was charged along with some glass beads to the carbon dioxide generating flask, *A*, of the modified Sakami (*9*) apparatus shown in Figure 1. The apparatus was evacuated and checked for leaks. When tight, flask *B* was immersed in liquid nitrogen and slow addition of concentrated sulfuric acid to flask *A* from reservoir *C* was begun (a glass wool plug inserted in the outlet of *A* prevented mechanical carry-over of sulfuric acid into *B*). Generation of carbon dioxide was completed in about 5 hours, flask *A* being finally filled with acid to displace all gas. Then 170 ml. of 0.38 *M* methyl magnesium iodide solution was gradually added from reservoir *D* to the solid $C^{14}O_2$ in *B*. *B* was intermittently removed from the bath and the entire

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The synthesis of DDT labeled with carbon-14 in the tertiary position was carried out in the following steps: barium carbonate to ethyl acetate to ethyl alcohol to chloral to DDT. Starting with 50 millimoles of barium carbonate containing 20 millicuries of activity, 15 grams of crude DDT were obtained (42% yield based on ethyl alcohol). Two crystallizations from ethyl alcohol yielded 6.11 grams of *p,p'*-DDT (17% yield) having a melting point of 107–107.5° C. The specific activity was approximately 0.5 millicurie per gram.

apparatus was shaken by hand during addition of the Grignard reagent. The course of the carbonation was followed roughly by manometer observations.

After all the Grignard reagent had been added, the contents of *B* were gradually allowed to warm up to about 0° C. and the apparatus was shaken thoroughly for about 20 minutes more. Finally the vacuum was relieved by introduction of dry nitrogen. The Grignard complex was decomposed by cautious addition of 50 ml. of 20% sulfuric acid while cooling in an ice bath. After thorough shaking, a slurry of 30 grams of silver sulfate in water was added and the mixture was shaken until all liberated iodide was precipitated. Flask *B* was then assembled with a condenser and steam generator and the product was steam-distilled, the ether being recovered and extracted with 1 *N* sodium hydroxide. The distillate was titrated with 1 *N* sodium hydroxide, 90% recovery of the acetic acid appearing in the first 500 ml. The distillation was continued until about 1800 ml. were obtained, the distillate showing no significant activity at that point. Of the Grignard carbonation apparatus described in the literature, the Sakami type is as simple as any, and yields of 90% or better can be obtained consistently with proper technique.

The distillate was evaporated by distillation of excess water, the concentrated product finally being filtered into a 1-liter round-bottomed distilling flask containing a layer of glass beads. Small but detectable losses of activity occurred during the process, although the distillate was distinctly alkaline throughout. As the evaporation approached the point of solidification of the sodium acetate, the flask was whirled to distribute the product in a thin layer on the walls of the flask and the surface of the glass beads. The last traces of water were removed by heating to 120° to 140° C. for 80 minutes while the flask was under high vacuum (10 to 20 microns of mercury); 3.73 grams of $CH_3C^{14}OONa$ (45.5 millimoles), 91% yield, were obtained.

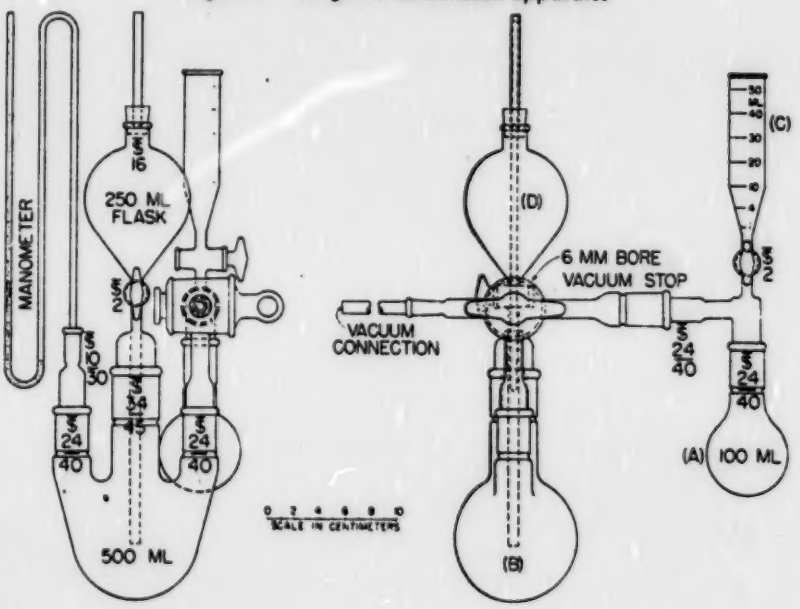
The acetate was converted to the ethyl ester by addition of 45 ml. of redistilled ethyl phosphate following the method of Ropp (*8*). The flask was assembled as shown in Figure 2. Trap *A* was immersed in an ice-salt bath and trap *B* in a dry ice–acetone bath. A Drierite tube was connected to the outlet of *B*. The flask contents were heated to 180° to 190° C. for about 4 hours under total reflux. After cooling, the outlet of *B* was attached to a high vacuum manifold and the dry ice–acetone bath was replaced with a liquid nitrogen bath. The system was then cautiously evacuated to about 13 microns of mercury and the flask contents were gradually raised to about 70° C. After cooling, the vacuum was relieved by introduction of dry nitrogen. The product in *B* was allowed to come to room temperature while being protected from atmospheric moisture. A yield of 5.4 ml. of ethyl acetate (not weighed) was obtained.

Reduction of $CH_3C^{14}OOC_2H_5$ To $CH_3C^{14}H_2OH$ The ethyl acetate was transferred to the reaction flask (*A*, Figure 3) using 15 ml. of redistilled diethylene glycol diethyl ether as transfer solvent, after the apparatus had been flushed with dry nitrogen. About 40 ml. of 2.0 *M* lithium aluminum hydride solution in diethylene glycol diethyl ether was gradually added to the ester solution in flask *A* from reservoir *B* while it was stirred magnetically and the flask maintained in an ice bath. Trap *C* was immersed in a dry ice–acetone bath with a Drierite tube attached to its outlet. After complete addition of the hydride solution, the reaction mixture was stirred for about 6 hours, being allowed to come to room temperature gradually. Sixty-five milliliters of β-phenoxyethyl alcohol were then gradually added from *B* while the reaction mixture was stirred. A slow stream of dry nitrogen was continually passed through the apparatus and trap *C* was immersed in liquid nitrogen. The reflux condenser, *D*, was cooled with refrigerated tap water. After about 8 ml. of the alcohol had been added, the initial vigorous reaction subsided and the mixture was gradually heated to 100° to 110° C. and maintained there for about 6 to 7 hours. The ethyl alcohol formed and recovered in trap *C* was directly distilled from the trap to free it from the small quantity of β-phenoxyethyl alcohol carried over from the reaction mixture.

A yield of 4.10 grams (89 millimoles) of $CH_3C^{14}H_2OH$ having a specific gravity of 0.82 was obtained. This represents an over-all yield of 89% based on barium carbonate. The yield is probably slightly high, as a small quantity of ethyl alcohol may have arisen from hydrolysis of ethylphosphate by traces of water during ethylation.

Chlorination of $CH_3C^{14}H_2OH$ The ethyl alcohol was transferred directly from the specially constructed receiver used in its distillation through a ball-joint connection to the chlorination vessel (*A*, Figure 4). *A* was immersed in liquid nitrogen and the closed receiver heated to transfer the last traces. Chlorine was passed through the alcohol at an average rate of 10 cc. per minute. The reflux condenser, *B*, was cooled with refrigerated water.

Figure 1. Grignard carbonation apparatus



Trap *C* was immersed in a dry ice–acetone bath to collect excess chlorine. No 'heat' was applied for the first 5 hours. An exothermic reaction takes place initially, which raised the temperature to a maximum of 41° C. during the first hour. During the second stage chlorine was passed through the reaction mixture at the same rate but at a temperature of 50° to 60° C. for 3 hours. Then the temperature was raised to 90° and held there for about 24 hours with the chlorine flow averaging between 5 and 10 cc. per minute. After cooling, nitrogen was passed through the system until the excess chlorine had been displaced. *C* was replaced by a tared receiver immersed in liquid nitrogen. The system was cautiously evacuated and steam was passed through the condenser. Finally *A* was heated to 90° to 95° C. for 10 to 15 minutes and then a flame was applied to the take-off lines to the trap. The system was allowed to cool under vacuum and then relieved with nitrogen. After thawing, weight and volume of the product were recorded; volume was 4.9 ml., weight 7.93 grams, and specific gravity 1.62.

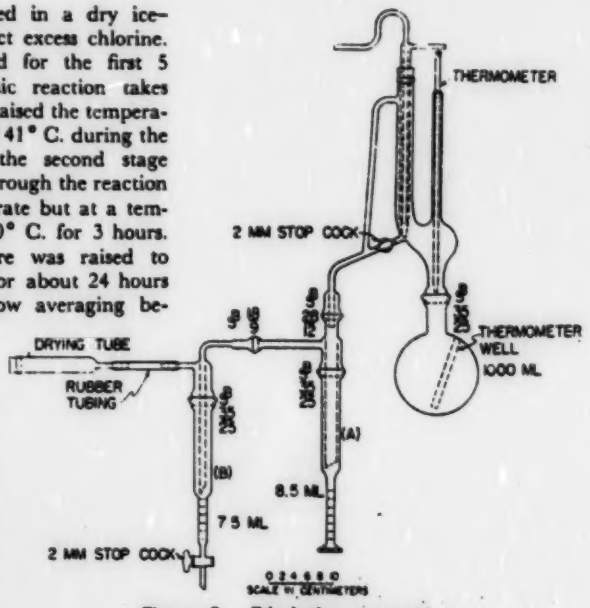


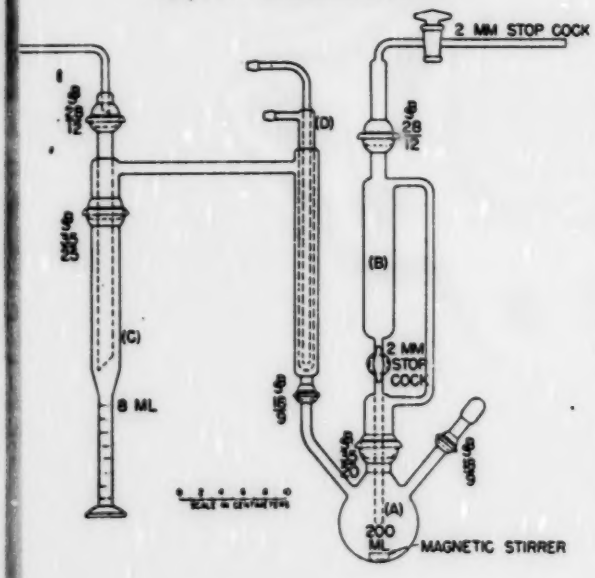
Figure 2. Ethylation apparatus

The long chlorination period is necessary to assure complete chlorination. Insufficient chlorination produces appreciable quantities of DDD in the final product, which is difficult to separate from DDT. The chlorinated product consists principally of chloral, chloral alcoholate, and some chloral hydrate, the latter depending on the freedom from moisture of the system. It is unnecessary to subject the product to further treatment, as it will readily condense with chlorobenzene under the proper conditions.

Preparation Of DDT

The extensive study of Mosher *et al.* (*6*) was followed in developing the best conditions for condensation of the chlorinated product with chlorobenzene. The product was transferred to a 3-necked 500-ml. reaction flask equipped with a stainless steel stirrer using 20 ml. (197 millimoles) of chlorobenzene as transfer solvent. Sixty milliliters of 99% sulfuric acid were added while the flask was immersed in an ice bath. With stirring and keeping the mixture cooled to 0° to 5° C., 60 ml. of 101% sulfuric acid were added intermittently, about 2 ml. every 10 minutes; a precipitate started separating out after addition of the first 15 ml. After complete addition of the acid, the reaction flask was packed in ice. Ten hours later the product was dissolved in *n*-hexane and the hexane solution was washed with 5% sodium carbonate and then with water. The solution was evaporated to dryness in a tared flask, heating to constant weight at 90° C. under vacuum. The crude DDT weighed 15.83 grams, representing a 42% yield based on ethyl alcohol. The product was recrystallized twice from ethyl alcohol, yielding 6.11 grams of *p,p'*-DDT, melting point 107–107.5° C., specific activity 0.46 ± 0.01 mc. per gram. This represents a 14% conversion of the carbon-14 of barium carbonate to DDT.

Figure 3. Reduction apparatus



The specific activity of the product was less than the calculated value, 0.56 mc. per gram, probably because of the presence of a small amount of ethyl alcohol in the ethyl acetate introduced by hydrolysis of ethyl phosphate during the ethylation of the sodium acetate.

The theoretical yield of DDT from 50 millimoles of barium carbonate by this synthesis is 100 millimoles, owing to the formation of 2 molecules of ethyl alcohol in the reduction of 1 molecule of ethyl acetate.

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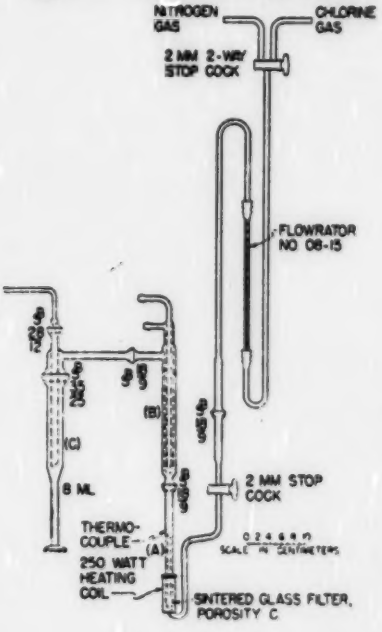
Received for review August 12, 1953. Accepted August 25, 1953. Presented before the Division of Agricultural and Food Chemistry, Pesticides Subdivision, at the 123rd Meeting of the AMERICAN CHEMICAL SOCIETY, *Los Angeles, Calif.*

Figure 4. Chlorination apparatus



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PUBLICATION

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Technology Branch
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Synthesis of Radioactive Parathion Using S^{35} [1,2]

By Jens A. Jensen and George W. Pearce

Received February 1, 1952

The insecticidal compound parathion, O,O-diethyl-O-*p*-nitrophenyl thiophosphate has been prepared and radioactively labeled with S^{35}. The following steps were used

$$S^{35} + PCl_3 \xrightarrow{AlCl_3} PS^{35}Cl_3 \quad (1)$$

$$PS^{35}Cl_3 + 2C_2H_5ONa \longrightarrow (C_2H_5O)_2\text{-}P(S^{35})\text{-}Cl + 2\,NaCl \quad (2)$$

$$(C_2H_5O)_2\text{-}P(S^{35})\text{-}Cl + NaO\text{-}C_6H_4\text{-}NO_2 \longrightarrow (C_2H_5O)_2\text{-}P(S^{35})\text{-}O\text{-}C_6H_4\text{-}NO_2 + NaCl \quad (3)$$

Phosphorus thiochloride was obtained by the method described by Knotz.[3] The cooled liquid was not washed but distilled directly. Reactions (2) and (3) followed those published by Fletcher, *et al.*[4-5] Seventy-five millimoles of S containing approximately 16 millicuries of activity yielded 44.4 millimoles of parathion (59%); sp. gr. 1.26 ± 0.01; $n^{35}D$ 1.5380; specific activity, 220 microcuries per millimole.

(1) From the Communicable Disease Center, Public Health Service, Federal Security Agency, Savannah, Georgia.

(2) For detailed paper order Document 3566 from American Documentation Institute, 1719 N Street, N.W., Washington 6, D. C., remitting $1.00 for microfilm (images 1 inch high on standard 35 mm. motion picture film) or $1.00 for photocopies (6 × 8 inches) readable without optical aid.

(3) Østerr F. Knotz, *Chem. Z.* **60**, 128 (1949).

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PUBLICATION

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TOXICOLOGY PROGRAM
Technology Branch
Communicable Disease Center
Atlanta 22, Ga.

[Contribution from Technical Development Laboratories, Communicable Disease Center, Public Health Service]

Chlorine Exchange between Aluminum Chloride and γ-1,2,3,4,5,6-Hexachlorocyclohexane

By George W. Pearce and Sonja Krause

Received July 23, 1956

Chlorine exchanges readily between aluminum chloride and γ-1,2,3,4,5,6-hexachlorocyclohexane (γ-HCC) at temperatures above 100° with some decomposition and isomerization, primarily to α-HCC and δ-HCC. Mechanisms for both exchange and isomerization are proposed.

Chlorinated hydrocarbon insecticides labeled with chlorine-36 would be very helpful in studies of the fate and toxicology of this class of compounds in resistant strains of arthropods as well as in higher animals. The use of exchange reactions appeared to offer a simpler way of preparing Cl-36 labeled compounds than their complete synthesis. In addition, study of exchange reactions can provide knowledge of the structural nature of compounds in relation to their properties in general. Using lindane, the γ-isomer of 1,2,3,4,5,6-hexachlorocyclohexane (γ-HCC), as a chlorinated hydrocarbon insecticide of importance chlorine exchange reactions between it and $AlCl_3$ labeled with Cl-36 have been attempted.

1,2,3,4,5,6-Hexachlorocyclohexane has previously been labeled by direct chlorination of benzene with chlorine-36.[1]

Experimental

Both the apparatus and the method of preparing labeled aluminum chloride were based on the work of Willard and colleagues[2,3] in which the reactions

$$HCl^* + AgNO_3 \longrightarrow AgCl^* + HNO_3$$

$$AgCl^* + Al \longrightarrow Ag + AlCl_3^*$$

are employed. The latter reaction is carried out under rigorously anhydrous conditions in an evacuated system. Two solutions of chlorine-36[4] one containing 41.6 mg. Cl/microcurie and the other 3.62 mg. Cl/microcurie, were available for this work. The silver nitrate was Mallinckrodt Reagent grade and the aluminum was Baker and Adamson, 16 to 60 mesh.

The AgCl was prepared in a tube protected from light and fused to a flask containing the γ-HCC[5] which was used without further purification. After the AgCl had been melted under vacuum to remove the last traces of water, Al was added, still under vacuum. When the mixture was heated to 460–500°, $AlCl_3$ was evolved as a gas and could be driven over into the flask containing the γ-HCC if this was cooled in liquid nitrogen. Heat had to be applied for one to two hours for complete conversion of the AgCl to $AlCl_3$. The flask containing $AlCl_3$ plus γ-HCC was then sealed off completely from the rest of the apparatus and could be treated as desired with the contents still under vacuum. A number of runs were made in which the heat treatment of the $AlCl_3$ and lindane and/or the shape of the reaction container were varied. Products from the reaction mixture were recovered by dissolving in ether and water and extracting each layer with small portions of the other solvent. Aliquots of the water phase and products obtained from the ether phase were assayed for Cl-36 activity after conversion to AgCl. A shielded end-window Geiger tube was used to count samples. The approximate disintegration rate was estimated by standardizing this count against a Q-gas flow counter which was assumed 50% efficient. Percentage exchange was calculated as percentage of total possible exchange at equilibrium.

Experimental Results

Run 1: 500 mg. lindane in 100-ml. reaction flask, 42.6 mg. Cl containing 1 microcurie. Lindane and $AlCl_3^{36}$ heated about 7 minutes at 115–130°; no discoloration m.p. 111.5–112.5° (recrystallized product); exchange, 16%.

Run 2: 500 mg. lindane in Y-shaped tube, 41.84 mg. Cl containing 11.5 microcuries. Lindane and $AlCl_3^{36}$ driven back and forth several times by heating alternate arms of

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(4) Cl-36-P Processed, Oak Ridge National Laboratory, AEC, Isotope Catalogue, July, 1952.

(5) Pure samples of α-, β-, γ-, δ- and ε-isomers were obtained from the Ethyl Corporation.

the reaction tube. Slight discoloration, m.p. 110–112° (recrystallized product); exchange, 5%.

Run 3: 500 mg. lindane in 100-ml. flask, 83.68 mg. Cl containing 23 microcuries. After melting the lindane several times by alternate flaming and cooling of the reaction flask, the reaction mixture was held in a melted condition at 112–115° for about 15 minutes by placing the apparatus in an oven. Extensive discoloration, m.p. 113–159° (recrystallized product). Melting point data indicated isomerization occurred.

Run 4: Same as run 3, except reaction mixture was subjected to less heat treatment and only a moderate amount of discoloration occurred. Melting point observations of the product again indicated some isomerization took place although not as extensively as in run 3.

Since isomerization appeared evident in runs 3 and 4, the products were chromatographed by the method of Harris[6]; the delta isomer was recovered by extracting the column with acetone.[7] Estimates of the activity and the percentage of the isomers isolated and their m.p.'s when possible are presented in Table I.

TABLE I

	Run 3			Run 4		
Isomer	%	M.p.° (recrys. product)	d./m./μg.[b]	%	M.p., °C.°	d./m./μg.[b]
α	65	153–155	43	10	103–108 (unrecryst. and before chromatographing)	35
γ	2	.....	50	90		2.2
δ and other products (column residue)	33	130–137	30	Trace		11

[a] Published m.p. of isomers involved: α 158°; γ 112.5°; δ 138°. [b] Disintegrations/minute/microgram.

Since isomerization occurred with the more severe heating a run was made in which the reactants (same as in runs 3 and 4) were held for 4 days at 70° in a 250-ml. flask. No discoloration occurred and the unrecrystallized product melted at 108.5–112.5° indicating no significant change in the lindane. A small but definite exchange amounting to 1 to 2% occurred.

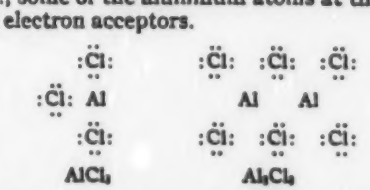
For the purpose of later discussion data for the d./m./μg. have been calculated for 100% exchange at the molar ratio of $AlCl_3$ and γ-HCC used in the experiments assuming all the chlorines of solid $AlCl_3$ exchange at the same rate. Under these conditions the d./m./μg. varies only with the number of HCC chlorines that may exchange. The calculated values are applicable to runs 3 and 4 since the same amount of $AlCl_3$ and γ-HCC was employed in these runs. If one chlorine exchanges from each molecule of HCC, the product should have 42 d./m./μg. Exchange of 2Cl yields 60 d./m./μg.; exchange of 3Cl yields 69 d./m./μg.; and exchange of all 6Cl yields 82 d./m./μg.

Discussion

The experimental data show that $AlCl_3$ exchanges chlorine readily with γ-HCC at temperatures at or above the melting point of γ-HCC, *i.e.*, 112.5°. Prolonged heating results in the isomerization of the γ-HCC mainly to α-HCC, with some formation of δ-HCC. It is the purpose of this discussion to propose a simple mechanism for both exchange and isomerization.

Willard and colleagues[2,3] have shown that $AlCl_3$ will exchange chlorine with organic compounds only while it is in the solid state. Exchange will not occur when the $AlCl_3$ is gaseous or dissolved.

If a molecule with empirical formula $AlCl_3$ existed, it would be a Lewis acid, an electron acceptor. However, it has been shown that both gaseous[8,9] and liquid[10] $AlCl_3$ exists as Al_2Cl_6 dimers, which have lost the electron-accepting properties of the hypothetical $AlCl_3$. Solid aluminum chloride, however, is in ionic form[11] and should retain some of the acidic properties of the hypothetical monomer, *i.e.*, some of the aluminum atoms at the surface will be electron acceptors.



When a molecule of γ-HCC approaches one of these electron acceptors, the unshared pair of electrons on one of the γ-HCC chlorines is probably attracted to that spot and may be held tightly enough to remove that chlorine from the molecule. As the rest of the molecule tumbles about the aluminum chloride surface, it soon picks up another chlorine ion to complete the exchange. Before considering the isomerization, it will be helpful to study Fig. 1 showing linear models of the HCC isomers.[12] The true configurations of these isomers correspond primarily to the "chair" form of cyclohexane. (The vertical lines represent the relative position of the chlorines.)

After a chlorine has been removed from a carbon of the γ-HCC molecule, it is probable that the three remaining bonds on that carbon should tend to become planar, thus giving the hydrogen a chance to "flip" over into the position formerly occupied by the chlorine. The "exchange" chlorine would then appear in the position formerly held by the hydrogen. It can be seen from Fig. 1 that the reversal of the chlorine at positions 2, 3, 5 or 6 will give one of the α-HCC mirror images. Positions 1 and 4 are considered later.

The data show that continued heating of the reactants causes more and more of the γ-HCC to convert to α-HCC; the α-HCC does not isomerize back to γ-HCC. Models of both isomers, constructed from Fisher–Hirschfelder models, illustrates a possible reason for this. Two of the chlorines in γ-HCC, in the 2,6-biaxial position, are situated so close to each other that their mutual repulsion is probably considerable. If either of these chlorines "flips over," α-HCC is formed. In order for the isomerization to reverse, the chlorine would have to return to the axial (higher energy) position.

The preceding mechanism requires that no α-HCC be formed without the exchange of at least one chlorine with the aluminum chloride. Appropriate evidence for this is shown in data for runs 3 and 4. In run 3, the α-HCC could be recovered pure, with activity 43 d./m./μg.; when one HCC chlorine has exchanged completely the activity should be 42 d./m./μg. (experimental error is probably about 10% or more). In this run the γ-HCC had 50 d./m./μg., while in run 4 it had only 2.2 d./m./μg. The impure α-HCC recovered in run 4, however, still had more than 35 d./m./μg. Thus, the minimum activity of the α-HCC is independent of the amount of γ-HCC that has had a chance to exchange.

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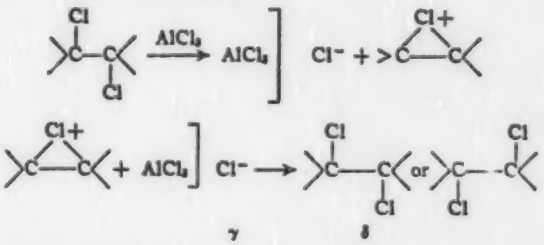
Although no pure δ-HCC was recovered, the evidence for the presence of this isomer is fairly conclusive. The absence of all other HCC isomers has not been established.

It is impossible to give a simple mechanism for the formation of the δ-HCC at this time; however, two possibilities can be discussed. First, it is possible that δ-HCC is formed from the α-HCC previously produced from the γ-HCC by the "flipping over" of chlorine 4. It is also possible that the delta isomer is formed from the gamma directly. When one of the 2,6-biaxial chlorines is removed, chlorine 1 is in the proper *trans* position to form a bridge between carbons 1 and either 2 or 6.[13] A chlorine ion may then be picked up in either of two positions as illustrated



Thus, either gamma or delta or mixtures of the two may result. It is, of course, possible that both mechanisms suggested occur.

In spite of the tendency for isomerization to occur in the chlorine exchange reaction between lindane and $AlCl_3$ sufficient quantities of labeled gamma isomer were obtained for use in tracer studies. The reaction is being studied further in an effort to arrive at conditions where isomerization can be kept to a minimum. Reagents other than $AlCl_3$ are also being considered as exchange media.

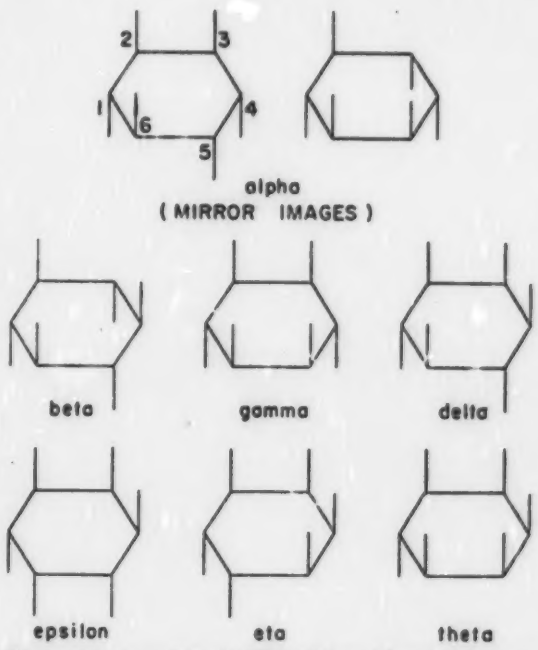


Fig. 1.—Linear models of 1,2,3,4,5,6-hexachlorocyclohexane.

Acknowledgment.—The authors wish to thank Dr. D. S. Noyce, University of California, who helped with nomenclature and clarified some of the ideas on mechanism presented.

SAVANNAH, GEORGIA

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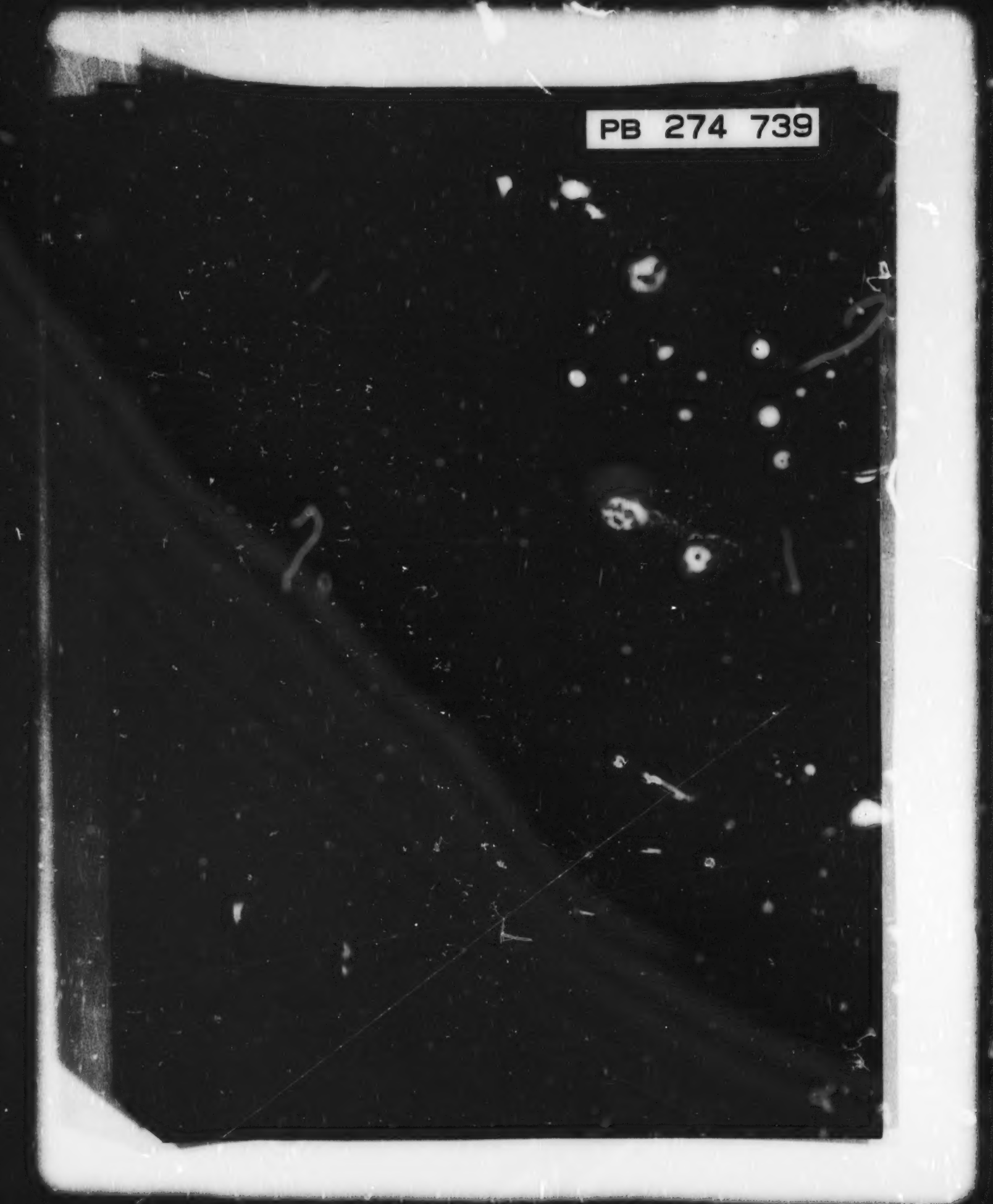
PB 274 739

On the Chemistry of the Isomeric Dichlorodiphenylacetaldehydes Related to DDT. Stereoelectronic Effects in the Hydrolysis of their Cyclic Acetals

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Received July 7, 1970[2]

The chemistry of the isomeric dichlorodiphenylacetaldehydes related to DDT was investigated to aid in delineating their possible role in the metabolism of this pesticide. In the case of the *p,p'*-isomer, the most important system under consideration, several literature syntheses were shown to be incorrect. The failure of at least one of these methods was attributed to the preferred oxidative involvement of the benzhydryl carbon via rearrangement of a β-hydroxy enol ether which normally led to the isolation of the *p,p'*-dichlorobenzophenone instead of the desired aldehyde. The *o,p'*-isomer exhibited chemistry similar to that of the *p,p'*-isomer although the steric effect of the ortho-substituted chlorine on the benzhydryl proton was notable. The *o,o'*-isomer was normal in that it did not undergo reactions involving the benzhydryl carbon. Some possible mechanistic and biological implications of the work are discussed.

La chimie des dichlorodiphénylacétaldéhydes isomères, apparentés au DDT, a été étudiée afin de délimiter leurs rôles possibles dans le métabolisme de ce pesticide. Dans le cas de l'isomère-*p,p'*, le système considéré comme le plus important, plusieurs synthèses données dans la littérature sont incorrectes. L'échec d'au moins une de ces méthodes est attribuée à l'entrée en jeu de l'oxydation préférentielle du carbone benzhydryle via une transposition d'un éther énolique β-hydroxylé qui normalement conduit au *p,p'*-dichlorobenzophénone au lieu de conduire à l'aldéhyde désiré. L'isomère-*o,p'* montre un comportement analogue à celui de l'isomère-*p,p'*, bien que l'effet stérique des substituants ortho sur le proton benzhydryle soit notable. L'isomère-*o,o'* a un comportement normal en ce sen qu'il ne subit aucune réaction impliquant le carbone benzhydryle. Certains mécanismes possibles et certaines implications biologiques de ce travail sont discutés.

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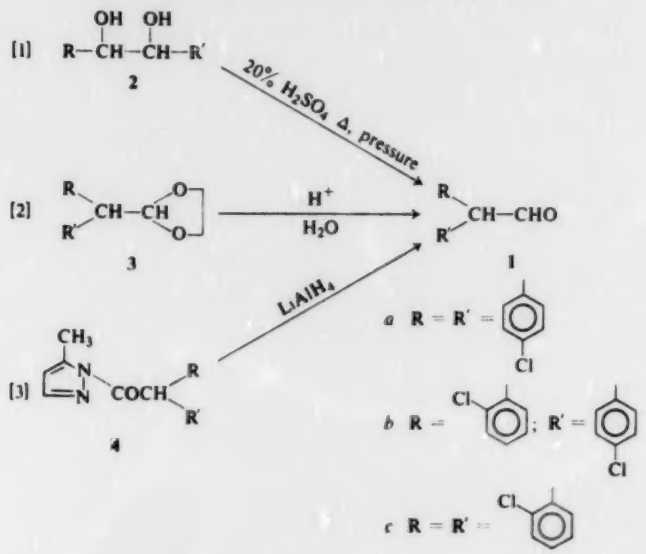
Today DDT is of immense biological importance, with vast quantities now distributed in the worldwide environment. However, surprisingly little work has been done toward gaining a firm theoretical understanding of its chemistry. The synthesis of the isomeric dichlorodiphenylacetaldehydes related to DDT and a study of some of their reactions were undertaken to help correct this deficiency. Investigation of their role in DDT metabolism (1) seemed essential since they are the logical intermediates in the formation of the dichlorodiphenylacetic acids which are major metabolites. Although several syntheses have been reported for these aldehydes, our attempts to reproduce some of them led to contradictory results which are theoretically interesting and may be of biological consequence.

The several syntheses reported for *bis*(*p*-chlorophenyl)acetaldehyde (**1***a*) include the rearrangement of 1,2-*bis*(*p*-chlorophenyl)ethylene glycol (**2***a*) in a sealed tube under vigorous conditions to effect a phenyl migration (2) (eq. 1); the hydrolysis of the cyclic acetal (3), 2-(*p,p'*-dichlorobenzhydryl)-1,3-dioxolane (**3***a*) (eq. 2); the reductive cleavage of the pyrazolene **4***a* (4) (eq. 3). In all three cases the aldehyde was reported as a crystalline compound varying in m.p. from 135 to 149 °C.

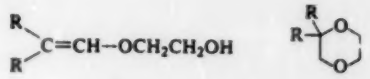
The synthesis of **1***a* by the hydrolysis of **3***a* appeared straightforward and was especially attractive since the starting material for synthesis of **3***a* was 2,2-dichloro-1,1-*bis*(*p*-chlorophenyl)-ethane (DDD) which is readily available. Our attempts (5) to reproduce this method afforded crystalline **3***a* whose structure confirmation conveniently eliminated structures **5***a* and **6** which are conceivable products of this reaction proposed by Riemschneider and co-workers. However, the results of our hydrolyses of **3***a* confirm that the previous workers (3) had isolated 4,4'-dichlorobenzophenone (**7**) instead of the desired aldehyde (**1***a*). The aldehyde was obtained as an oil which could not be crystallized. The crystalline material isolated by the previous workers (2–4) is characteristic of **7** in varying degrees of purity. It was later found that other workers (6) had arrived at the same conclusion since their

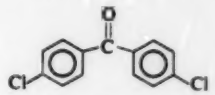
[1]Chamblee Toxicology Laboratory, Environmental Protection Agency, Chamblee, Georgia 30341.

[2]Revision received August 31, 1971.



Darzens synthesis of the aldehyde had afforded an oily product. Investigation of this chemistry was undertaken since the chemistry encompassing the formation of the dichlorobenzophenone might have a bearing on its occurrence in DDT metabolism.



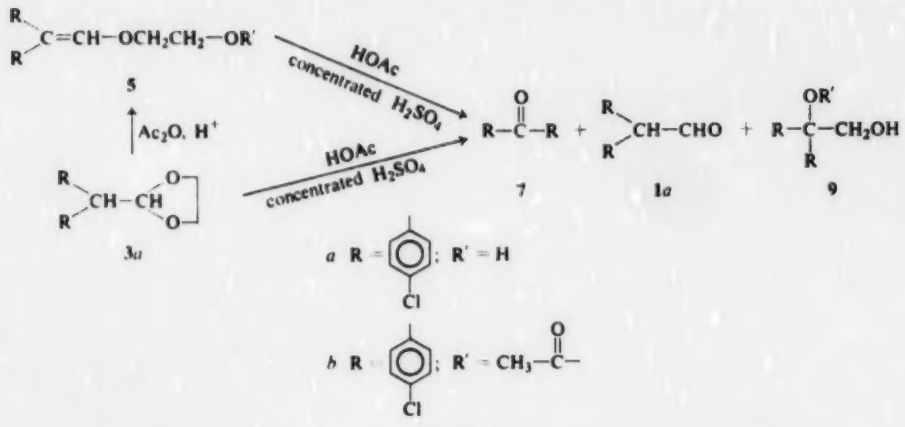


Based on these findings, it seemed plausible but mechanistically obscure that **1***a* might be intermediate in the formation of **7** during the hydrolysis of the acetal **3***a* under the heterogeneous conditions. However, the aldehyde was recovered in good yield when it was subjected to the conditions of hydrolysis with no appreciable conversion to dichlorobenzophenone (**7**). When the hydrolysis was carried out using mixtures of *p*-dioxane and 20% aqueous HCl (1:1 v/v), **7** was formed as the major characterizable product (30–60% yields) in 3–5 h at reflux temperature. There was no t.l.c. evidence for the intermediate formation of **1***a*. This result was supported by following the hydrolysis of **3***a* by n.m.r. under similar conditions.[3] Since the purity and structure of **3***a* have been rigorously established, an alternative route by which dichlorobenzophenone **7** can be formed from acid hydrolysis of acetal **3***a* without invoking the intermediacy of aldehyde **1***a* apparently exists. The experiments outlined in Scheme 1 were carried out with the objective of trapping or stablizing intermediates which might be responsible for the formation of **7** from acetal **3***a*.

Solvolysis of **3***a* in acetic acid in the presence of concentrated sulfuric acid at 100 °C for 1 h gave a mixture of the dichlorobenzophenone **7**, aldehyde **1***a*, and the acetoxyethanol **9***b*. These products were isolated by preparative t.l.c.[4] and

[3]The hydrolysis was carried out at 75° in *p*-dioxane-d_8 with 20% DCl. The n.m.r. scans were made at 5-min intervals for 1 h. The acetal apparently underwent immediate hydrolysis; however, there were no resonances indicating the formation of **1***a*. A control experiment had indicated that **1***a* was stable under these conditions.

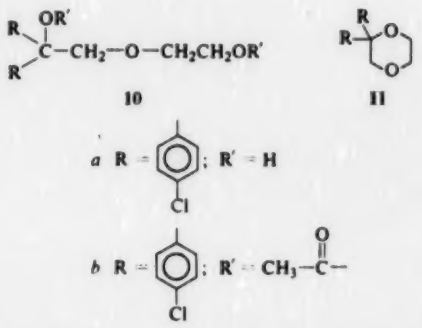
[4]The acetoxyethanol **9***b* decomposed on t.l.c. to two materials; one remaining at the origin and the other near the solvent front. The latter material characteristically fluoresced light blue under short wave u.v. light. The exact nature of these products will be discussed later; however, n.m.r. studies of product mixtures of **9***b* in the presence of the n.m.r. shift reagent (**7**), $Eu(DPM)_3$, confirmed that the acetate group (three proton, singlet) was associated with the benzhydryl carbon and a hydroxyl group was associated with the methylene group (two proton, singlet) since the latter group experienced the largest paramagnetic shift with the reagent while the aromatic signals experienced relatively little shift.



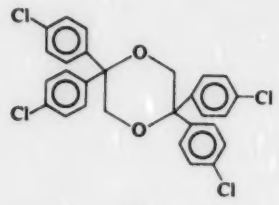
SCHEME 1

identified by their spectral properties. The benzophenone **7** constituted about 10–15% of the total along with major amounts of the aldehyde and acetoxyethanol. Solvolysis of the enol ether acetate **5***b*, which was obtained from **3***a* in good yield by treatment with acetic anhydride containing concentrated sulfuric acid, gave qualitatively but not quantitatively the same product mixture; the benzophenone **7** was formed in about 40% yield with appreciable amounts of the acetoxyethanol **9***b* and trace amounts of aldehyde **1***a*. It was also shown that neither **1***a* nor **9***b* were intermediate in the formation of **7** under the solvolysis conditions.

Since the enol acetate **5***b* affords **7** as a major product and the acetoxyethanol **9***b* is not intermediate in this conversion, the benzophenone **7** must have resulted from rearrangement of **5***b* itself or from some other yet undetected intermediate. Protonation of the enol ether acetate at the ether oxygen should activate the molecule to attack by acetate ion at the benzhydryl carbon or intramolecular attack by its own acetate group via a *quasi*-six-membered ring. The former possibility should have resulted in the diacetate **10***b*. However, this compound was not found among the products and suggested that **10***b*, if formed, possibly undergoes further cleavage at the ether oxygen to form the acetoxyethanol **9***b*, a major product of the acetic acid solvolysis reaction. Nevertheless, 2,2-*bis*-(*p*-chlorophenyl)-diethylene glycol (**10***a*) was synthesized according to the reported method (8), and its hydrolysis properties were investigated. The diethylene glycol could dehydrate in a cyclic fashion (9) to



form dichlorobenzophenone **7** or dehydrate to 2,2-*bis*(*p*-chlorophenyl)-*p*-dioxane (**11**) which could possibly undergo pyrolysis to **7**. Hydrolysis experiments with **10***a* repeatedly gave mixtures as determined by t.l.c. and gas chromatography (g.c.). The mixtures consisted primarily of two materials whose chromatographic properties were reminiscent of the decomposition products[4] of the acetoxyethanol **9***b*; one material was highly polar and polymeric in nature while the other material was relatively non-polar and fluoresced light blue under u.v. light. The latter material was purified by preparative t.l.c. and identified as the symmetrical *p*-dioxane **12**. The n.m.r. spectrum of **12** revealed the non-equivalency of the methylene protons, and Drieding models show that because of the steric requirements of the bulky phenyl groups the molecule prefers a twist-boat conformation in which the methylene protons experience different environments. The mass spectrum of the compound contained a parent ion greater than 500 (*m/e*)

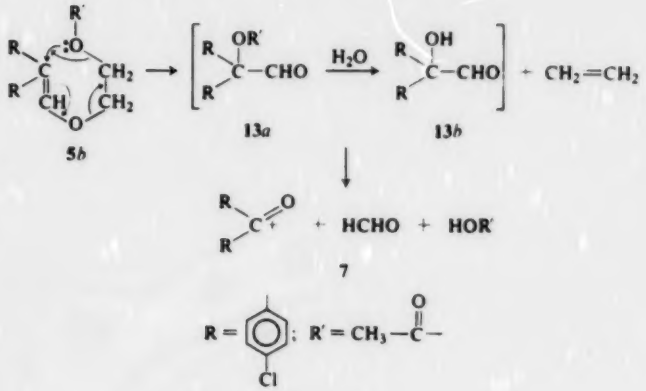


12

and was otherwise indicative of structure **12**. The dioxane **12** and polymeric products were also shown to be spontaneous decomposition products of the diethylene glycol **10***a* with or without solvent. The most plausible explanation for formation of such products would be that intramolecular hydrogen bonding may facilitate decomposition of the diethylene glycol **10***a* to the ethylene glycol **9***a* which in turn can undergo dimerization to **12** or polymerization. The dichlorobenzophenone **7** was only a minor decomposition product (<10%) of **10***a* and was detected by electron capture g.c. A small amount of dioxane **11** was also detectable among the products. Therefore, the intermediacy of **10***b* in the solvolysis of the enol ether acetate **5***b* seems likely since it would explain the presence of the acetoxyethanol **9***b* and polymeric material among the products. The formation of dichlorobenzophenone **7** from rearrangement of **5***b* is now credible and is shown in Scheme 2.[5] Invoking the intermediacy of an α-hydroxy aldehyde (**13***b*) in the formation of the dichlorobenzophenone **7** was attractive since such an intermediate would be expected to be both chemically and thermally labile and is a conceivable product in the other erroneous syntheses reported for aldehyde **1***a* by other workers. Although there is no chemical evidence to support the intermediacy of **13***a*, gas chromatography – mass spectrometry (g.c.–m.s.) confirmed its presence among the decomposition products of the enol ether acetate **5***b*. Gas chromatography on a 3% diethylene glycol succinate/2% orthophosphoric acid column at 170 °C gave eight peaks for pure enol ether acetate **5***b*, six of which were identified by their mass spectra. The first peak was the expected dichlorobenzophenone **7** in comparatively good yield having a base peak of *m/e* 139 $(R—C\equiv O)^+$ in its mass spectrum. The second peak was identified as the α-hydroxy aldehyde (**13***b*) whose mass spectrum also has a base peak of *m/e* 139 along with M (*m/e* 281), M-1 and M-HCHO (*m/e* 251) of intensities approximately 0.3% that of the base peak. The third peak was the expected aldehyde **1***a* in less than 10% yield of that of the ketone **7**. The fourth peak was a major component and was identified as the α-acetoxy aldehyde (**13***a*) although the parent molecular ion (*m/e* 313) was not discernible. However, there are prominent fragments corresponding to the loss of both HCHO (*m/e* 293) and HOAc (*m/e* 264) as well as the base peak of *m/e* 139. The presence of these fragments in the spectrum implies that both the aldehyde and acetate moieties must have been present in the original molecule. The detection of the parent ion of the α-hydroxy aldehyde (**13***b*) is probably a reflection of increased stability of this ion via hydrogen bonding. Both peaks five and seven were too weak to identify conclusively. Peaks six and eight were identified as the enol ether alcohol (**5***a*) and the enol ether acetate (**5***b*) respectively. The acidic column conditions favored decomposition since g.c. on a 3% SE-30 column at 212 °C gave smaller relative amounts of the major decomposition products. In fact, the α-acetoxy aldehyde (**13***a*) was not detectable under these conditions; however, this may also be a reflection of the poorer stability of acetate **13***a* under these conditions since the α-hydroxy aldehyde was detected. In addition, the introduction of the enol ether acetate **5***b* into the mass spectrometer by the direct probe inlet produced spectra indicating that extensive rearrangement had occurred.

In light of these data, it was suspected that similar intermediates could occur in the hydrolysis of **3***a* under aqueous acidic conditions affording dichlorobenzophenone **7**. Hydrolysis of the substituted dioxane **12** and the enol ether acetate **5***b* in *p*-dioxane and 20% HCl (1:1 v/v) gave the aldehyde **1***a* as the major product. These results are not entirely surprising since dioxane **12** might be expected to undergo cleavage and rearrangement to the aldehyde on heating with a mineral acid and, in the case of **5***b* cleavage at the ether oxygen would be expected

[5]The nature of the products from this reaction seems to clearly indicate that the normal oxonium ion resonance contribution of enol ethers, $R_2\overset{\ominus}{C}CH=\overset{\oplus}{O}—R'$, is of little importance in **5***b*. Rearrangement of **5***b* can be considered analogous to the thermal rearrangements of enolic ethers in general and allyl aryl ethers (Claisen rearrangement) producing in the case of **5***b* somewhat unexpected products because of the anomalous electronic distribution. Such thermally influenced rearrangements of this type for the most part are known (10) to proceed via first-order kinetics as depicted for **5***b* although it was not ascertained.



SCHEME 2

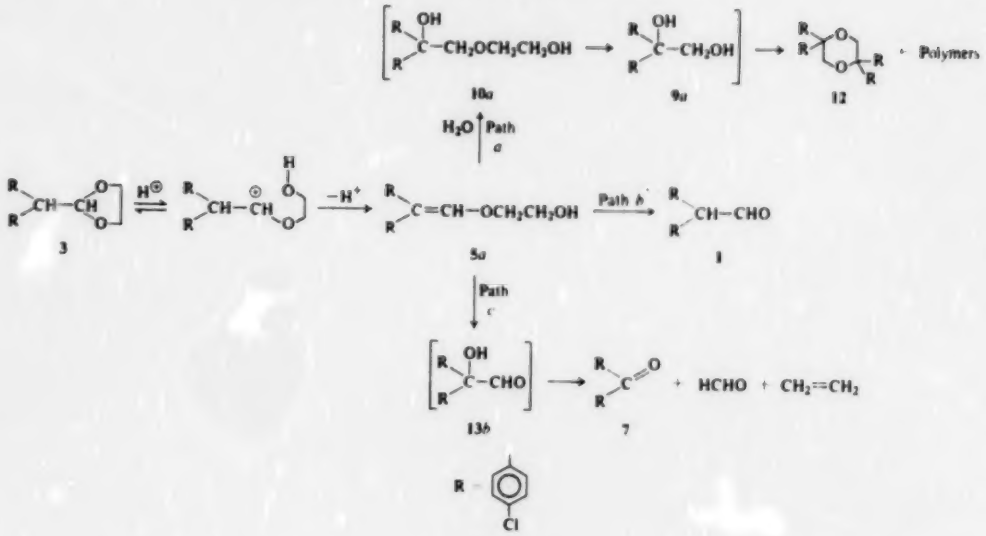
to occur more rapidly than hydrolysis of the acetate group such that an appreciable amount of the suspected "real" intermediate, the enol ether alcohol (**5***a*) is never available for rearrangement leading to dichlorobenzophenone **7**. In considering the transition state for rearranging **5***b* to **7**, the comparatively large acetate group in the vicinity of the reacting center restricts the number of configurations which may be assumed by the atoms involved, resulting in further reduction in entropy which is rate-retarding. Therefore, one would expect the enol ether alcohol (**5***a*) to rearrange as shown in Scheme 2 with a more positive entropy which would be comparatively rate-enhancing. The fact that the enol ether alcohol could not be isolated is consistent with this argument.

Variations[6] in the hydrolysis conditions of acetal **3***a* gave qualitatively but not quantitatively the same products, acetaldehyde **1***a* and dichlorobenzophenone **7**. Hydrolysis in 95% ethanol with 20% aqueous sulfuric acid (2:1 v/v) produced approximately equal quantities of **7** and **1***a*. Hydrolysis in 95% ethanol or 1,4-dioxane with concentrated sulfuric (10:1 v/v) afforded aldehyde **1***a* in good yields. Totally aqueous acidic conditions gave either no reaction or the dichlorobenzophenone **7** as the major product. These results imply that more aqueous conditions and hence more heterogeneous conditions favor production of **7**. Under more heterogeneous conditions solvolysis reactions would be minimized and intramolecular vapor phase reactions, which would be thermally influenced, become more important. This is consistent with a cyclic decomposition of **5***b* to **7** as delineated in Scheme 2. The intermediates formed in the hydrolysis of acetal **3***a* which are most tenable with our results are given in Scheme 3.

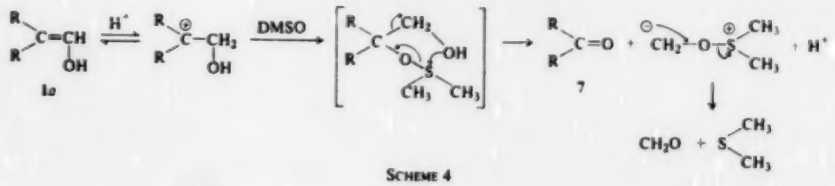
Preequilibrium protonation of **3** followed by decomposition to a resonance-stabilized carbonium ion would be expected (11). The enol ether alcohol (**5***a*) could be formed by deprotonation of this carbonium ion, and once formed, can undergo several reactions (paths *a*–*c*). Normal hydrolysis as in path *b* would lead to aldehyde **7**. Protonation of the enol ether oxygen followed by hydration of the double bond (path *a*) would lead to diethylene glycol **10***a* which in turn could decompose to ethylene glycol **9***a* ultimately leading to dimers and polymers. Protonation of the ether oxygen followed by intramolecular attack on the double bond and rearrangement would lead to the α-hydroxy aldehyde **13***b* which would decompose[7] to the dichlorobenzophenone **7**.

[6] Several classical methods for preparing aldehydes (Rosenmund and metal hydride reductions of the acid chloride, chromium oxide oxidation of the alcohol, etc.) were unsuccessful for **1***a*; in most cases the major isolable product was the dichlorobenzophenone **7**. The mechanistic implications of these results are not completely understood.

[7] The expected by-products of these disproportionation reactions are formaldehyde and ethylene. One would expect formaldehyde to polymerize under the reaction conditions, and attempts to trap it with various carbonyl derivatizing agents were unsuccessful. Likewise, ethylene could not be trapped as its dibromide; however, an n.m.r. hydrolysis study of **3***a* with aqueous D_2SO_4 in d_6-dimethylsulfoxide, which indicated the presence of **7** as well as trace amounts of **1***a*, showed a singlet of weak intensity at a chemical shift (τ 4.55) identical to that of authentic ethylene.



SCHEME 3



SCHEME 4

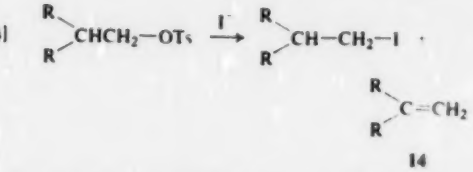
Our investigations were extended to include the *o,p'*-dioxolane **3***b* since commercial preparations of DDT contain the *o,p'*-isomer as a major component whose metabolism is qualitatively similar although quantitatively different (12). Dioxolanes **3***a* and *b* are electronically analogous and would be expected to undergo similar reactions. Under the hydrolysis conditions of **3***a* that afford only minor amounts of the expected aldehyde **1***a*, **3***b* affords quantitative yields of aldehyde **1***b*.

The anomalous hydrolysis of **3***a* seems to be manifested in the facility by which the benzhydryl carbon of this system can undergo oxidation. This reflects greater charge stabilization associated with the bis(*p*-chlorophenyl) system than with the *o,p'*-system which must be explained on the basis of steric arguments. CPK-Models[8] indicate that there are two sterically derived factors which would hinder or prevent oxidation of the benzhydryl carbon in the *o,p'*-system (**3***b*). There is steric hindrance to removal of the benzhydryl proton imposed by the *o*-chlorine,[9] and there is steric inhibition of resonance which would impair charge stabilization once the proton has been removed. Aldehyde **1***b* would be the expected hydrolysis product of **3***b* regardless of which of the two steric effects is more important in determining the course of the reaction.

Previous investigators (6) observed steric dependence in attempting to prepare the isomeric iodides from the corresponding tosylates of the

[8]Corey–Pauling–Koltun atomic models.

[9]A measure of this effect is evident from a comparison of the chemical shift of the benzhydryl proton in *p,p'*-DDT (τ 5.00) with that in *o,p'*-DDT (τ 4.25) and *o,p'*-DDD (τ 4.28); an ortho chlorine within the aromatic ring deshields the benzhydryl proton more than a para-substituted chlorine. See Keith *et al.* (13) for a discussion of the n.m.r. spectra of DDT-related compounds.

[4] 

14

dichlorodiphenylethanols (eq. 4). The elimination product **14** was the sole product in the case of *p,p'*-isomer whereas the *o,p'*-isomer gave a mixture of the two products. On the other hand, the *o,o'*-isomer gave the iodide as the sole product. Steric factors are known (14) to play an important role in bimolecular elimination reactions. Steric hindrance to resonance stabilization of the product might also influence the reaction course in this case.

A steric dependence of this system was further demonstrated in our laboratory by observing the n.m.r. spectra of the aldehydes in dimethylsulfoxide (d_6-DMSO). In DMSO, **1***a*, is converted to its enol form almost quantitatively upon solution as evidenced by a decrease in the n.m.r. proton signal of the benzhydryl carbon. In most instances this enolization was accompanied by a small (< 10%) conversion to the dichlorobenzophenone **7**. Crystalline material isolated from the DMSO solutions had properties identical to those of authentic **7**. The *o,p'*-isomer undergoes a slower rate of enolization as measured by n.m.r.; however, there was no evidence to indicate reactions with DMSO. It was shown using the *o,p'*-isomer that the enolization rate in DMSO could be catalyzed by the addition of either acid or base. Shaking the protio-aldehyde in chloroform-*d* with D_2O in the presence of sodium carbonate resulted in the exchange of the benzhydryl proton for deuterium while the aldehyde proton remained unchanged. The *o,o'*-isomer[10] **1***c*, however, failed to undergo enolization in DMSO at temperatures ranging from 26 to 75 °C. In comparison, diphenylacetaldehyde enolizes in DMSO to a lesser extent than did the bis(*p*-chlorophenyl)acetaldehyde. From this result one infers that the electronic effects of the chlorine substituents as well as steric factors play a role in the enolization processes. In the case of the *p,p'*-isomer, the electronic effects must also influence subsequent reactions since the dichlorobenzophenone **7** was detected in its DMSO solutions. Since enolization of **1***a* appears to be very rapid in DMSO, it is probable that formation of **7** involves the enol. Steric hindrance to removal of the benzhydryl proton would be manifested if enolization were required to form a reactive species. Although there is no compelling evidence, the following pathway is proposed (Scheme 4).[11] Preequilibrium protonation of the enol could generate the bis(*p*-chlorophenyl) carbonium ion system which is susceptible to attack by oxygen of DMSO. The resulting labile alkoxysulfonium salt could undergo intramolecular attack at sulfur to afford **7**.

This work has shown that the bis(*p*-chlorophenyl) system in conjunction with a vicinal electron deficient carbon, such as is contained in the aldehyde moiety and possibly in a trichloromethyl group,[12] can undergo rather facile reactions involving the benzhydryl carbon usually resulting in an overall oxidation at this position which may be concomitant with the loss of the adjacent group. In comparing the oxidative propensities of the *p,p'*- and *o,p'*-systems, it is evident that the requisite removal of the benzhydryl proton is subject to the stereoelectronic requirements imposed by the chlorophenyl groups. The *o*-chlorophenyl compounds have much less tendency toward oxidation and double bond formation at their benzhydryl carbons. In light of this work, the possible intermediacy of the α-hydroxy aldehyde **13***b* in the formation of dichlorobenzophenone **7** during DDT metabolism is plausible. The ease of enolization of the *p,p'*-aldehyde **1***a* could facilitate its interactions in biological systems subsequently leading to redox products such as α-hydroxy aldehyde **13***b*. Our results also lend some credence to the corollary that, if such an aldehyde were formed *in vivo*, conjugates[13] involving the hydroxy group could activate the

[10]The aldehyde **1***c*, which was obtained from Aldrich Chemical Co., Milwaukee, Wisc., exhibited no unusual reaction properties.

[11]The use of polarizing agents to promote DMSO oxidations of alcohols has been reported (15) to produce alkoxysulfonium salts which undergo base-catalyzed decomposition to carbonyl compounds. The hydroxymethylalkoxysulfonium ion which could be formed from **1***a* may constitute a special case of this in which the hydroxymethyl group acts as an internal base to effect the stepwise decomposition to two carbonyl compounds.

[12]The compound 1,1-bis(*p*-chlorophenyl)-2,2,2-trichloroethanol has been shown (16) to pyrolyze to **7** and chloroform.

[13]Previous workers (17) have shown that DDT metabolite conjugates are formed, although their exact nature is not known. However, they do not appear to be conjugated with glucuronic or sulfuric acids.

molecule as an alkylating agent.[14] Nevertheless, the occurrence of such an aldehyde would implicate a host of new and interesting metabolites which will be described elsewhere. Although, it is not surprising that the aldehyde **1***a* has not been detected in DDT metabolism, earlier work (18) has shown that 2,2-bis(*p*-chlorophenyl)-ethanol (DDOH), a known DDT metabolite (1), is a substrate of crystalline liver alcohol dehydrogenase which oxidizes DDOH to **1***a*.

Experimental

Melting points were taken on a Fisher–Johns melting point apparatus and are corrected. The i.r. spectra were obtained with Perkin–Elmer 337 and 521 spectrophotometers. The n.m.r. studies were made with a Varian T-60 and an HA-100 spectrometer equipped with a variable temperature probe using tetramethylsilane (TMS) or sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) as internal references. Routine gas chromatograms were obtained with a Varian Aerograph model 1868-40 chromatograph equipped with a tritium detector. The low-resolution mass spectra were obtained with an LKB 9000 gas chromatograph-mass spectrometer. Thin-layer chromatograms were run on Uniplate, Silica Gel GF plates 250 and 500 μ in thickness and were developed in methylene chloride. The bands were detected with u.v. light.

Formation of 2,2-Bis(p-chlorophenyl)acetaldehyde (1a)

(a) From Darzens Condensation

To a solution of 7.5 ml (0.077 mol) of ethyl chloroacetate and 10 g (0.04 mol) of 4,4′-dichlorobenzophenone (Eastman Organic Chemicals) in 25 ml of anhydrous benzene under nitrogen was added 6.0 g (0.12 mol) of sodium hydride (50% dispersion) with stirring over a 2 h period. The reaction mixture was stirred for 16 h at room temperature and poured into 50 ml of ice-cold 25% HCl solution. The resulting suspension was extracted with two 50-ml portions of benzene. The combined extracts were dried over anhydrous sodium sulfate and evaporated to dryness. The partially solidified residue was recrystallized from methanol, affording 9.5 g of ethyl β,β-bis(*p*-chlorophenyl)glycidate melting at 98–105°. Without further purification this material was dissolved in 40 ml of ethanol and the resulting solution was refluxed for 6 h with 15 ml of 35% aqueous potassium hydroxide solution. The ethanol was removed by rotary evaporation and the remaining mixture was diluted with 25 ml of distilled water and extracted with two 30-ml portions of ether. The aqueous phase was acidified (p*H* 4–5) with 20% hydrochloric acid, forming a milky solid suspension. The suspension was extracted with two 50-ml portions of ether and the combined extracts were washed with distilled water, dried over anhydrous sodium sulfate, and evaporated to an oily residue. Decarboxylation of the crude material was accomplished by heating in an oil bath at 140–160° under reduced pressure (water aspiration) for 30 min. The crude product **1***a* weighed 4.5 g (60%). The aldehyde was purified by dissolving in 10 ml of methanol and adding the resulting solution dropwise with stirring to 25 ml of saturated sodium bisulfite solution. The mixture was stirred at room temperature for 5 h and the solid suspension was removed by filtration. The cake was washed with water, 2-propanol, and then ether before drying under vacuum. The aldehyde was regenerated prior to use by heating portions of the bisulfite addition product with 10% sodium carbonate solution at 75° until an oil separated (15–30 min). The pure aldehyde was obtained by extraction into ether and had the following spectral properties: i.r., λ (neat) 5.80 (C=O), 3.49 (CH), 13.2 (C—Cl); n.m.r., τ (chloroform-*d*) 0.10 (doublet, J = 2.4 Hz, 1H, CHO), 3.00 (multiplet, 8H, aromatics), 5.08 (doublet, J = 2.4 Hz, 1H, benzhydryl).

The *p*-nitrophenylhydrazine derivative prepared according to the method of Shine (19) contained one molecule of methanol of crystallization and melted at 85–88°. When this material was recrystallized from ether – petroleum ether, the methanol was removed to give a crystalline compound melting at 135–138 °C: i.r., λ (Nujol) 3.03 (NH), 6.26 (C=N), 7.58, 7.72 (NO_2); n.m.r., τ (dimethylsulfoxide-d_6) 1.84, 2.95 (pair of doublets, J = 9 Hz, for *p*-nitrophenyl aromatics); 2.10, 4.82 (pair of doublets, J = 6 Hz, for azomethinyl and benzhydryl protons); 2.57 (multiplet for bis(*p*-chlorophenyl) aromatics).

The *p*-nitrophenyl hydrazone of the dichlorobenzophenone **7** was prepared according to the same procedure and melted at 213–215°: i.r., λ (Nujol) 3.02 (NH), 6.30 (C=N), 7.55, 7.90 (NO_2); n.m.r., τ (dimethylsulfoxide-d_6) 1.97 (doublet, J = 9 Hz, 2H of *p*-nitrophenyl aromatics); 2.64 (multiplet, 2H of *p*-nitrophenyl aromatics, 8H of bis(*p*-chlorophenyl) aromatics).

(b) From Hydrolysis of 2-(4,4′-Dichlorobenzhydryl)-1,3-dioxolane (3a)

To a solution of 85 mg (0.28 mmol) of dioxolane in 5 ml of 95% aqueous 1,4-dioxane was added 0.4 ml of concentrated H_2SO_4. The resulting solution was magnetically stirred and refluxed for 3 h. The 1,4-dioxane was removed by rotary evaporation and the aqueous phase doubled in volume with distilled water to 3 ml. The aqueous solution was then extracted with two 3-ml portions of anhydrous ether and the combined extracts were dried over anhydrous Na_2SO_4 containing solid Na_2CO_3. Evaporation of the ether extracts under a nitrogen stream followed by pumping *in vacuo* gave 79 mg of a mixture composed of the 2,2-bis(*p*-chlorophenyl) acetaldehyde **1***a* and starting material. The aldehyde which was purified by preparative t.l.c. (40 mg, 55% yield) was identical in every respect to aldehyde obtained via the Darzens synthesis. Longer reflux times (6–8 h) gave increasingly higher yields of aldehyde under these conditions.

Solvolysis of 2-(4,4′-Dichlorobenzhydryl)-1,3-dioxolane (3a) in Acetic Acid

To a solution of 8 ml of glacial acetic acid and 0.05 ml of concentrated H_2SO_4 was added 155 mg (0.5 mmol) of

[14]The formation of the symmetrical dioxane **12** previously discussed can be considered a head-to-tail alkylation of two molecules of the acetoxyethanol **9***b* via displacement of the acetate groups. This reaction appears to be facile since it occurs directly on a silica gel t.l.c. plate during development. This compound was also found as a reaction product of unsuccessful attempts to form amino acid Schiff bases of aldehyde **1***a*.

dioxolane **3a**. The resulting solution was heated at 100 °C for 1 h. The acetic acid was removed by lyophilization and the residue pumped *in vacuo* over P_2O_5. The residual oil was dissolved in chloroform-*d* and examined by n.m.r. spectroscopy. The spectrum indicated a mixture of products of which the three major components were tentatively identified as 2,2-bis (*p*-chlorophenyl)acetaldehyde (**1a**), 4,4′-dichlorobenzophenone (**7**), and 1,1-bis(*p*-chlorophenyl)-1-acetoxyethanol (**9b**) in an approximate ratio of 3:1:3. Preparative t.l.c. of the mixture gave 45 mg of aldehyde **1a** and 15 mg of dichlorobenzophenone **7** along with 27 mg of dimer **12** and 20 mg of polymeric material (dark brown material remaining at the origin) both of the latter materials being decomposition products of the acetoxyethanol **9b**. A small amount (10 mg) of pure dimer **12** was obtained by repeated preparative t.l.c. and had the following properties: i.r., λ ($CHCl_3$ film) (aromatic) 6.70, 12.2; (C—O—C) 8.88, 9.18, 9.85; n.m.r., τ (chloroform-*d*) 2.99 (multiplet, aromatics), 4.65, 5.89 (doublets, J = 2.0, Hz, methylene protons); τ (benzene-d_6) 3.10 (multiplet, aromatics), 5.19, 6.17 (doublets, J = 2.0, Hz, methylene protons).

Anal. Calcd. for $C_{28}H_{20}O_2Cl_4$: C, 63.42; H, 3.77. Found: C, 63.19, H, 4.38.

1,1-Bis(p-chlorophenyl)-3-oxa-1-pentenyl-5-acetate (Enol Ether Acetate (5b))

To a solution of 20 ml of acetic anhydride and 2 ml of concentrated H_2SO_4 was added 2.0 g (65 mmol) of the dioxolane **3a**. The resulting solution was heated at 100 °C with stirring for 0.5 h. On cooling, the reaction mixture was poured into ice water (approximately 30 ml) and extracted with two 50-ml portions of anhydrous ether. The combined extracts were washed with saturated Na_2CO_3 solution, water, and then dried over anhydrous Na_2SO_4 followed by evaporation. The residue remaining (approximately 3.2 g) was chromatographed on a column of Silcar CC-7 (40 g) using a mixture of hexane:chloroform:methanol (3:2:1) as the eluting solvent. The fractions were analyzed with t.l.c. and combined accordingly. The residue remaining on evaporation of the solvent weighed 1.87 g (91%). A portion of this material was crystallized twice from *n*-hexane affording a crystalline hygroscopic solid melting at 73–75 °C: i.r., λ (neat) (C=O) 5.75, (O—C=C) 6.20; n.m.r., τ (chloroform-*d*) 2.76 (multiplet, aromatics); 3.54 (singlet, enolic proton); 5.80 (multiplet, methylene protons); 7.97 (singlet, acetate CH_3).

Anal. Calcd. for $C_{18}H_{16}O_3Cl_2$: C, 61.56; H, 4.56; O, 13.77; Cl, 20.22. Found: C, 61.22; H, 4.74; O, 13.58; Cl, 20.04.

Formation of 4,4′-Dichlorobenzophenone (7)

(a) From Hydrolysis of 2-(4,4′-Dichlorobenzhydryl)-1,3-dioxolane (3a)

To a mixture of 10 ml of 1,4-dioxane and 10 ml of 20% aqueous HCl was added 1.0 g (3.2 mmol) of dioxolane **3a**, and the resulting mixture was refluxed for 3.5 h. The solution after cooling to room temperature was diluted with additional water to 50 ml and extracted with two 30-ml portions of anhydrous ether. Combined ethers were washed with water, dried over anhydrous Na_2SO_4, and then evaporated. The residue was dissolved in 10 ml of methanol and gave on prolonged cooling 0.21 g (30%) crystalline material identical in every respect to authentic 4,4′-dichlorobenzophenone **7**. The mother liquor was evaporated and examined by n.m.r. spectroscopy. The remaining material (0.52 g) consisted of a mixture of dioxolane **3a**, dichlorobenzophenone **7**, and aldehyde **1a** (1:1:2). The total yield of **7** was approximately 57%.

(b) From Solvolysis of Enol Ether Acetate (5b)

A solution of 3 ml of glacial acetic acid and 0.1 ml of concentrated H_2SO_4 containing 83 mg (0.24 mmol) of enol ether acetate was heated at 100 °C for 1 h. The acetic acid was removed by lyophilization and pumping *in vacuo* over P_2O_5. The residual oil was examined by n.m.r. spectroscopy and consisted primarily of 4,4′-dichlorobenzophenone (**7**) and 1,1-bis(*p*-chlorophenyl)-1-acetoxyethanol (**9b**). The oil was transferred to preparative t.l.c. plates by streaking, and the plates were developed in methylene chloride. Three major bands were detected and the middle band was removed (24 mg, 40%) and found to be identical in every respect to 4,4′-dichlorobenzophenone (**7**). The other two bands were indicative of the dimer and polymeric decomposition products of the acetoxyethanol **9b** and were not isolated; a band was found at the origin and one near solvent front which had light blue fluorescence under short wave u.v. light.

2-(o,p′-Dichlorobenzhydryl)-1,3-dioxolane (3b)

To a solution of 6.9 g (0.028 mol) of 1-(*o*-chlorophenyl)-1-(*p*-chlorophenyl)-2,2-dichloroethane in 25 ml of ethylene glycol was added 7.05 g (0.051 mol) of powdered anhydrous potassium carbonate. The mixture was refluxed at 190–200° for 16 h and poured into ice water. The aqueous suspension was extracted with two 25-ml portions of ether, and the combined extracts were dried over anhydrous sodium sulfate and taken to dryness. The crude residue (6.2 g) afforded 4.0 g (59%) of crystalline material melting at 73–76° when crystallized from methanol: n.m.r., τ (chloroform-*d*) 2.80 (8H multiplet for aromatics); 4.54, 5.30 (2H pair of doublets, J = 4 Hz, benzhydryl proton and methinyl proton of dioxolane ring); 6.26 (4H multiplet, dioxolane ring).

2-(o-Chlorophenyl)-2-(p-chlorophenyl)acetaldehyde (1b)

A solution of 9.4 g (0.034 mol) of **3b** in 25 ml of *p*-dioxane and 50 ml of 25% aqueous sulfuric acid was refluxed with stirring for 5 h. The reaction mixture on cooling was extracted with two 50-ml portions of ether. The combined ether extracts were extracted twice with dilute sodium bicarbonate solution and washed twice with distilled water. The ether phase was dried over anhydrous sodium sulfate and evaporated to a liquid residue weighing 9.2 g. The liquid was purified by formation of the bisulfite addition product as described for **1a** except the stirring time was increased to 24 h. A 7.0 g portion of the addition product when decomposed with dilute sodium carbonate solution gave 5.0 g (81%) of pure aldehyde **1b** with the following spectral properties: i.r., λ (neat) 5.75 (C=O), 6.65 (aromatic), 13.3 (C—Cl); n.m.r., τ (chloroform-*d*) 0.16 (singlet, 1H, CHO), 2.80 (multiplet, 8H, aromatics), 4.67 (singlet, 1H, benzhydryl).

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Synthesis and Reactions of a Proposed DDT Metabolite, 2,2-*bis*(*p*-Chlorophenyl)acetaldehyde

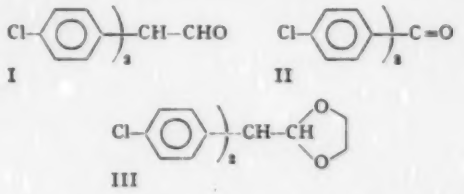
Studies of the metabolism of DDT led to the preparation of 2,2-*bis*(*p*-chlorophenyl)acetaldehyde (I) in order to investigate the possibility of its intermediacy[1] in DDT metabolism. Results of attempts to reproduce a reported synthesis[2,3] of I have shown that both this report and an even earlier report[4] of a different synthetic approach are incorrect. It was observed in this laboratory that I undergoes oxidative deformylation under mild oxidizing conditions to form the benzophenone (II). In comparison, the *o,o'*-isomer was quite stable under the same conditions. These results are of theoretical interest and may have biological significance.

Materials and methods. Melting points were taken on a Fisher-Johns melting point apparatus and are corrected. IR-spectra were obtained with Perkin-Elmer 521 and Beckman IR-12 spectrophotometers. The NMR studies were made with a Varian A-60 spectrometer equipped with a variable temperature probe using TMS or TMS[5] as internal reference. The gas chromatograms (GC) were obtained with a Varian Aerograph 1525 B chromatograph equipped with a tritium detector and employing a polar column of 1% orthophosphoric acid and 3% diethylene glycol succinate as the liquid phase and 30/60 Chromasorb W as the stationary phase. The low resolution mass spectra were obtained with an LKB 9000 gas chromatograph-mass spectrometer (GC-MS). Thin layer chromatograms (TLC) were run on Eastman Chromagrams, Type 6060, with fluorescent indicator and were developed in hexane-chloroform-methanol (3:2:1). The spots were detected with UV-light; Rf (I) 0.59, Rf (II) 0.71. Synthetic methods are given in the following discussion.

Results and discussion. The reported[2] method involved the treatment of 2,2-dichloro-1,1-*bis*(*p*-chlorophenyl)-ethane with sodium hydroxide in refluxing ethylene glycol to form the cyclic acetal, 2-(*p,p'*-dichlorobenzhydryl)-1,3-dioxolane (III). The product obtained by these workers after hydrolysis with refluxing 20% hydrochloric acid and recrystallization melted at 147 °C. Acceptable elemental analyses were given for the product and for its *p*-nitrophenyl-hydrazone (PNPH) derivative, but no spectral data were given.

Our attempts to reproduce the method yielded the crystalline acetal (III) whose structure was confirmed by its IR-, NMR- and mass-spectra. The NMR-spectrum is most diagnostic with a pair of doublets arising from the coupling (J = 4 cps) of the benzhydryl proton (5.82 τ) with the methinyl proton (4.57 τ) of the dioxolane ring.

However, hydrolysis of III gave an oil which could not be crystallized. Thin layer chromatography of the oil indicated that there were 2 major components, neither of which was starting material III. Gas chromatography confirmed that the oil was a mixture of 2 materials which were obtained in variable yields. The low resolution mass-spectrum of each component in the oil was obtained with a GC-MS combination. A study of the spectra indicated that the GC peak with the longest retention time gave a mass-spectrum expected for the desired aldehyde (I).



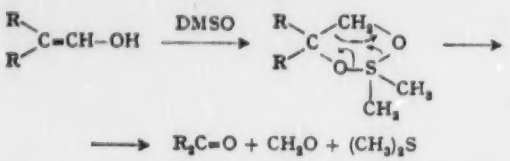
However, the mass-spectrum of the other GC peak had a parent ion which was 14 mass units lower than the parent ion of I and indicated the presence of two chlorine atoms in the molecule. Further analysis of this spectrum suggested that *p,p'*-dichlorobenzophenone (II) was the other major component in the hydrolysis product. An authentic sample of II was obtained and subjected to GC-MS analysis and was found to have an identical GC retention time and superimposable mass-spectrum to that of the unidentified component in the oily hydrolysis product. Crystalline material which had properties identical to those of II was later obtained from the oily hydrolysis product by slow crystallization from methanol. The melting point of authentic II (145 °C) and its PNPH (213 °C) derivative were almost identical to those reported[2] (147 and 211 °C, respectively) for I and its PNPH derivative and indicated that the earlier work was in error.

It was observed that the aldehyde could be separated from II by formation of its bisulfite addition product. Control experiments indicated that the ketone was virtually unreactive toward a saturated sodium bisulfite solution. The aldehyde was obtained as a viscous liquid on decomposition of the addition product with dilute aqueous sodium carbonate solution and was identified by its chromatographic and spectral properties[5]. Vacuum distillation of I is not recommended, since the presence of II was always detectable in the distillate. The thermal

[1] J. E. Peterson and W. H. Robison, Toxicol. appl. Pharmac. *6*, 321 (1964).
[2] R. Riemschneider, I. Arhle, W. Cohnen and E. Heilmann, Chem. Ber. *92*, 900 (1959).
[3] E. F. Riener, U.S. Patent 2,834,708 (1958).
[4] *Beilsteins Handbuch der organischen Chemie*, 4th Ed. *I*, VII, p. 439.
[5] The NMR of I in $CDCl_3$ showed doublets (J = 2.4 cps) at 0.10 τ (CHO) and 5.08 τ (*p*-Cl O_2-CH). The aromatics appeared as a multiplet centered at 3.00 τ.

lability[6] of the aldehyde was further evidenced when repeated injections of I of the same concentration gave varying amounts of II at the expense of I on the acidic polar column used for its GC analysis. The decomposition is probably catalyzed by the orthophosphoric acid additive used to shorten retention times and eliminate tailing.

Attempts to obtain an NMR-spectrum of I in dimethysulfoxide (DMSO) resulted in appreciable decomposition of I into II. This result was confirmed by TLC and GC of the isolated product. However, the electronically analogous 2,2-*bis*(*o*-chlorophenyl)acetaldehyde did not undergo decomposition in DMSO at temperatures ranging from 26–75 °C. Steric hindrance to removal of the benzhydryl proton which is greatest in the *o,o'*-isomer and smallest in the *p,p'*-isomer may explain this result. This steric effect would be manifested if enolization were required to form a reactive species, i.e.



$$\longrightarrow R_2C{=}O + CH_2O + (CH_3)_2S$$

The integrated NMR-spectra of I in d-chloroform and d_6-acetone indicated that I obtained directly from decomposition of the bisulfite addition product has a purity greater than 95%, which was sufficient for subsequent reactions. Aldehyde obtained in this manner was derivatized with *p*-nitrophenylhydrazine according to the method of SHINE[7] and the product was recrystallized from methanol-water as reported earlier[8]. A compound containing one molecule of methanol of crystallization melting at 85–88 °C was obtained and when this product was recrystallized from ether-petroleum ether, the methanol was removed to give a crystalline compound melting at 135–138 °C. Both derivatives were chromatographically identical. The IR-, NMR- and mass-spectra of this product were those expected of the desired aldehyde derivative. When an oily product obtained on hydrolysis of III was treated directly with PNPH according to the reported method, the highly insoluble benzophenone derivative crystallized directly from the reaction mixture while the soluble aldehyde derivative was obtained only after laborious preparative TLC of the mother liquor. These results confirm our belief that the previous workers had isolated ketone instead of I.

Based on these findings, it seemed plausible but mechanistically obscure that I might be intermediate in the formation of II during the hydrolysis of the acetal (III) under the heterogeneous conditions. However, significant amounts of aldehyde were recovered when it was subjected to the conditions of hydrolysis. When the hydrolysis was carried out under homogeneous conditions using *p*-dioxane with aqueous HCl, II was formed as the major product in 1 h at reflux temperature with no TLC evidence for the intermediate formation of I. This result was supported by following the hydrolysis of III by NMR under similar conditions[9].

Although a thorough study of the solvent effects on the mechanism of hydrolysis of III was not made, several solvents were tried in an attempt to improve on the yield of aldehyde. None of these variations in the hydrolysis procedure affording ketone gave a significant increase in the yield of I. An alternative approach to the synthesis of I, which gave fair yields but was more reproducible than hydrolysis of III, was a glycidic ester condensation of II and ethyl chloroacetate. Recent workers[9] also used the Darzen's reaction for the successful preparation of I which was patterned after a successful report[10] of its use to prepare 2-(*o*-chlorophenyl)-2-(*p*-chlorophenyl) acetaldehyde. Aldehyde (I) obtained in our laboratory from this reaction sequence was purified via its bisulfite addition product and was identical in every respect to I obtained via hydrolysis of III.

PERRY et al.[11] in 1965 have found II as an isolable metabolite of DDT which was believed to have been derived from 2,2-*bis*(*p*-chlorophenyl)-acetic acid (DDA). It was observed in our laboratory that DDA does not give rise to II in vitro under the same conditions by which I affords II. On the other hand, I is a logical precursor of DDA. Furthermore, previous work[1] has shown that 2,2-*bis*(*p*-chlorophenyl) ethanol (DDOH) can be obtained from in vivo transformations of other known DDT metabolites. Therefore, the occurrence of II, DDA and DDOH in DDT metabolism is strong supporting evidence for the intermediacy of I.

Greater biological significance may possibly be derived from the inherent reactivity of I as an aldehyde. For example, reaction of I with amino acids would form Schiff bases which might be incorporated into proteins thus chemically binding DDT residues and simulating storage. Therefore, the fate of I depends on whether or not it is enzyme bound and its proximity to stabilizing functional groups which may or may not be bound. However, regardless of what its fate may be, decomposition to form II, oxidation to form DDA, or carbonyl derivatizations with various nucleophiles, its detection in vivo will be difficult because of its instability and reactivity[12].

Zusammenfassung. Es wird eine Methode beschrieben, nach der authentischer 2,2-*bis*(*p*-chlorphenyl)acetaldehyd erhalten wird. Die Charakterisierung dieses Aldehyds hat gezeigt, dass die in der Literatur bisher erschienen Angaben unrichtig sind. Der Aldehyd geht unter milden oxydativen Bedingungen unter Deformylierung in 4,4-Dichlorbenzophenon über. Mögliche biologische Bedeutung dieser Reaktionen werden diskutiert.

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Atlanta Toxicology Branch, Bureau of Science, Food and Drug Administration, Consumer Protection and Environmental Health Service, Public Health Service, U.S. Department of Health, Education, and Welfare, Atlanta (Georgia 30333, USA), 2 May 1969.

[6] A vapor phase IR-spectrum of the aldehyde at elevated temperatures indicated that it apparently undergoes decomposition to *bis*(*p*-chlorophenyl)methane.

[7] H. J. SHINE, J. org. Chem. *24*, 252 (1959).

[8] The hydrolysis was carried out at 73 °C in *p*-dioxane-d_8 with 20% DCl. The NMR was scanned every 5 min for 1 h. The acetal apparently underwent immediate hydrolysis; however, there were no resonances indicating the formation of I. A control experiment had indicated that I was stable under these homogeneous conditions.

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[11] A. S. PERRY, S. MILLER and A. J. BUCKNER, J. agric. Food Chem. *11*, 457 (1963).

[12] We gratefully acknowledge the assistance of Mr. ROBERT HAWK in obtaining the mass-spectra of the compounds.

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PUBLICATION

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TOXICOLOGY PROGRAM
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ISOTOPE-LABELED INSECTICIDES

Synthesis of Carbon-14–Labeled Aldrin and Dieldrin

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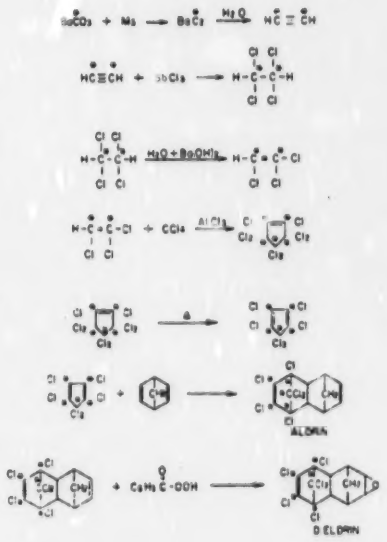
The insecticides 1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydro-1,4-*endo*-exo-5,8-dimethanonaphthalene (aldrin) and 1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,-8a-octahydro-1,4-*endo*-exo-5,8-dimethanonaphthalene (dieldrin) were prepared labeled with carbon-14. They were synthesized by labeling hexachlorocyclopentadiene and subsequent reaction with 2,5-norbornadiene. Starting with $BaC^{14}O_3$, a 28% yield of aldrin (melting point 102.5° C.) and a 22% yield of dieldrin (melting point 181° C.) were obtained. The specific activities were 3.6 and 3.5 ± 0.1 mc. per gram for aldrin and dieldrin, respectively.

During studies at this laboratory on the fate of aldrin and dieldrin in susceptible and resistant strains of insects and in higher animals it became evident that the chemical methods now available for these compounds are not adequate, especially when an attempt is made to account fully for the dosage applied. It was obvious that C^{14}-labeled aldrin and dieldrin would be a great aid in our studies and, accordingly, their synthesis was undertaken. The present paper is a report on this work.

Aldrin is ordinarily prepared by the Diels-Alder reaction of hexachlorocyclopentadiene with 2,5-norbornadiene (bicyclo[2.2.1]hepta - 2,5 - diene), and dieldrin is prepared by the peracid oxidation of aldrin. The problem was to prepare either hexachlorocyclopentadiene or 2,5-norbornadiene with a C^{14} label. Synthesis of C^{14}-labeled hexachlorocyclopentadiene appeared to be the most promising approach.

The following six- and seven-step reaction scheme was used in the synthesis of C^{14}-labeled aldrin and dieldrin:



Carbon-14 labeled acetylene was prepared by a modification of the method of Cramer and Kistiakowski (*2*). The yields were approximately 70%, as determined by gas volumetric measurements with inactive runs.

The labeled acetylene was converted to tetrachloroethane by treatment with a mixture of antimony pentachloride and trichloride. This reaction as given by Krall (*3*) was found to give very low yields. After the acetylene had been complexed with the antimony pentachloride, it was necessary to decompose the complex by heating for a much longer period of time than that used by Krall to obtain satisfactory yields. With inactive acetylene, 85% yields were consistently obtained in this reaction.

Trichloroethylene was prepared by refluxing the tetrachloroethane with barium hydroxide and steam-distilling the product. Yields of 85 to 90% were obtained with inactive trichloroethylene.

Octachlorocyclopentene was prepared from the trichloroethylene by the method of Prins (*4*). This reaction gave a 70% yield.

The dechlorination of octachlorocyclopentene to hexachlorocyclopentadiene and the subsequent conversion to aldrin and dieldrin were based on

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methods described by Burton, Potter, and Silverman (*1*). The dechlorination was found to be essentially quantitative and the Diels-Alder reaction gave 70 to 75% yields. Oxidation of aldrin to dieldrin gave a 63% yield after two crystallizations.

Experimental

Tetrachloroethane, $C^{14}HCl_2C^{14}HCl_2$. A mixture of 0.59 gram of barium carbonate containing 39 mc. of activity and 19.5 grams of inactive barium carbonate was mixed with 48.6 grams of 70- to 80-mesh magnesium. This was placed in a stainless steel reaction bomb, which later was used as the acetylene generator (*A*, Figure 1). The bomb was sealed with a copper gasket and screw cap fitted with inlet and outlet tubes. The reaction was carried out under a helium atmosphere, the bomb being heated at 725° C. for 15 minutes.

After cooling to room temperature the screw cap of the bomb was replaced by another screw cap with a male 40/50 standard-taper joint and rubber O-ring. This was immediately put into position as the acetylene generator (*A*, Figure 1). The system (Figure 1) was purged with dry nitrogen to remove moisture and oxygen before the liquid nitrogen Dewar flasks were put in place. After the flasks were put in position, the nitrogen flow was reduced to a rate that was just perceptible, as evidenced by the bubbles produced in the oil (*B*, Figure 1) at the end of the train.

A total of 200 ml. of water was very carefully added through the dropping funnel (*C*, Figure 1) over a 2-hour period, followed by the careful addition of 500 ml. of concentrated hydrochloric acid over another 2-hour period. The acetylene generator was kept cold throughout the addition of water and acid with an ice water bath. The contents were finally refluxed for 15 minutes to drive over the last traces of acetylene. The acetylene was condensed in the liquid nitrogen–cooled traps (*D*, *E*, Figure 1), while any moisture not condensed in the water-cooled condenser was retained by the ice water–cooled trap or the silica gel drying tube (Figure 1).

For chlorinating the acetylene, a solution of 12.0 grams of antimony trichloride and 89.7 grams of antimony pentachloride was placed in the reaction flask (*F*, Figure 2). Traps *D* and *E* with the acetylene were attached to the vacuum train as shown in Figure 2. With *D*, *E*, and *F* immersed in liquid nitrogen, the entire system (Figure 2) was evacuated to 30-micron pressure.

The acetylene was then vacuum-transferred to the chlorinating flask, *F*. With stopcocks *A'* and *B'* closed and *C'*, *D'*, and *E'* open, the acetylene–antimony chloride mixture was allowed to warm up to room temperature. The

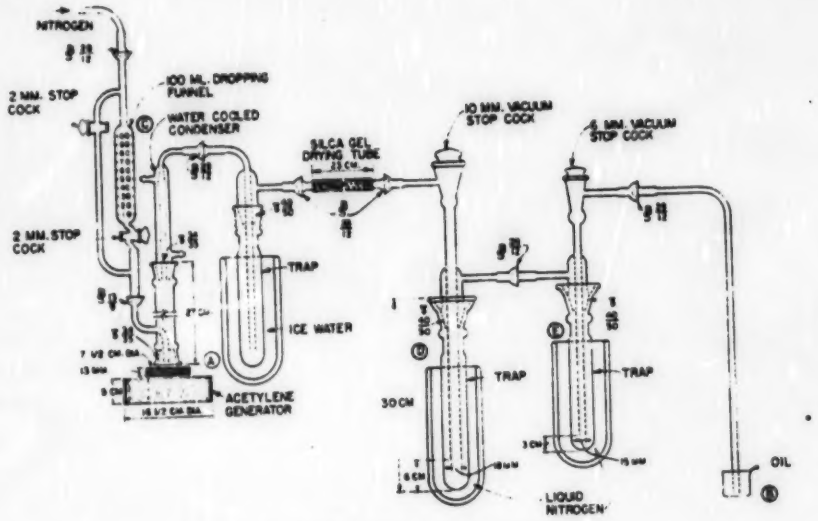

Figure 1. Acetylene generation apparatus

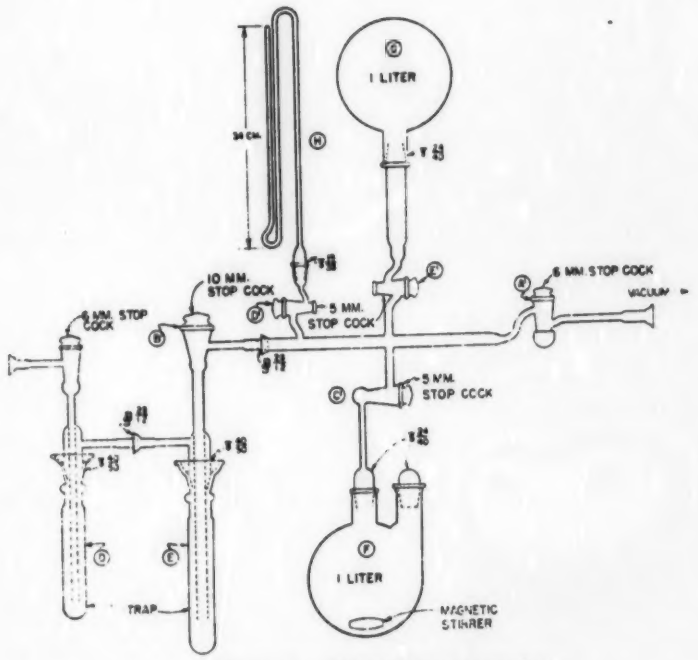

Figure 2. Chlorination apparatus

purpose of flask *G* is to add sufficient volume to the system to keep it well below atmospheric pressure. The pressure indicated by manometer *H* at this point was 28 cm., so it was safe to confine the gas in a smaller volume.

The liquid nitrogen bath was replaced on flask *F*, and the acetylene was frozen onto the antimony chloride mixture. With stopcocks *C'* and *D'* open and all other stopcocks closed, the liquid nitrogen bath was removed from flask *F* and replaced by an ice water bath. When the mixture became liquid, it was stirred magnetically and the progress of the absorption of acetylene was followed by the pressure readings. A minimum pressure of 4.4 cm. was reached after 1 hour and the absorption was assumed to be complete.

The liquid nitrogen was again replaced on flask *F* and the remaining gas was frozen. Stopcock *C'* was closed and the reaction mixture was held at 100° C. on a steam bath for 16 hours to bring about complete decomposition of the acetylene–antimony pentachloride complex to produce tetrachloroethane.

The assembly (Figure 2) was modified by removing the 1-liter flask, *G*.

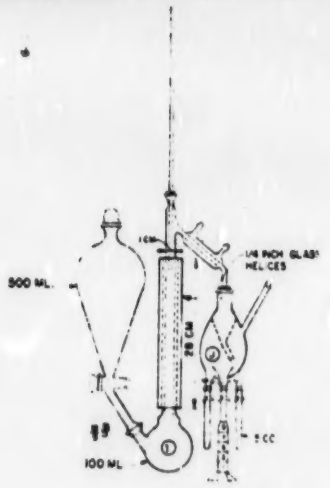

Figure 3. Distillation apparatus

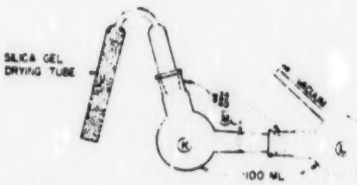

Figure 4. Recrystallization apparatus

Traps *D* and *E* were replaced by a ball joint stopper and the stopper in flask *F* was replaced by a 2-necked adaptor with standard-taper joints. A tube containing Ascarite was placed in one neck of the adaptor to serve as a vent, and an Erlenmeyer flask containing 70 grams of calcium hydroxide was attached to the other neck by means of a flexible rubber tube.

An ice bath was placed around flask *F*, and 200 ml. of water were slowly added to the reaction mixture with stirring. This was followed by the careful addition of 70 grams of calcium hydroxide to hydrolyze the antimony chlorides. The tetrachloroethane and some water were then separated from the reaction mixture by direct steam distillation. About 30 ml. of distillate were collected.

Trichloroethylene. Tetrachloroethane was dehydrochlorinated by refluxing with water and barium hydroxide. The receiving flask for the steam distillation served as the reaction flask. An additional 40 ml. of water and 10.0 grams of barium hydroxide octahydrate were added to the reaction flask.

The reaction mixture was refluxed for 5 hours and the trichloroethylene and some water were then distilled and collected in a receiver. The yield was 2.5 ml. or 3.7 grams. This is about 56% of the theoretical yield based on barium carbonate.

Octachlorocyclopentene. The synthesis of C^{14}-labeled trichloroethylene, starting from barium carbonate, was repeated on the same scale and the two batches were combined for a total of 7.4 grams. The trichloroethylene was dried over 6 grams of silica gel and then transferred with the aid of carbon tetrachloride to a 250-ml. dropping funnel. Carbon tetrachloride was added to give a total volume of 170 ml. Two grams of freshly sublimed aluminum chloride and 30 ml. of carbon tetrachloride were placed in a 500-ml., two-necked, round-bottomed flask. The 250-ml. dropping funnel was attached to one neck and a reflux condenser with a drying tube was attached to the other neck.

The reaction mixture was refluxed gently and stirred with a magnetic stirrer throughout the reaction. The trichloroethylene in carbon tetrachloride was added dropwise over a 9-hour period. This was followed by the dropwise addition, over a 2.5-hour period, of 1.74 grams of inactive trichloroethylene in 50 ml. of carbon tetrachloride. The reaction mixture was refluxed for one additional hour and, after cooling, it was filtered with suction through a bed of Celite on a Büchner funnel and received directly in a 500-ml. dropping funnel. The solution was washed three times with 30-ml. portions of water and then mounted in position on the distilling flask, *I*, as shown in Figure 3.

The carbon tetrachloride was removed by flash distillation at atmospheric pressure. When all of the solvent was removed, the fraction cutting adaptor, *J*, was put in position and the product was distilled under reduced pressure. A low-boiling forerun was collected below 75° C. at 0.75-mm. pressure. The fraction distilling above 75° C. at 0.75 mm. was collected as octachlorocyclopentene. A small amount of tarry residue remained in the distilling flask, *I*, at the end of the distillation.

The yield of crude octachlorocyclopentene was 5.4 ml. or 9.89 grams. This was vacuum-transferred to flask *K* of the recrystallizing apparatus shown in Figure 4. Methanol (10 ml.) was added and the product was solidified by cooling in a dry ice–acetone bath and broken up into a fine powder with the use of a glass rod. The methanol was removed while still cold by tilting the apparatus so that the receiver *L* was in an upright position and applying a vacuum. The octachlorocyclopentene was retained on the sintered-glass disk, *M*. The yield of octachlorocyclopentene was 8.68 grams or 72% based on trichloroethylene. This is 44.5% based on barium carbonate.

Hexachlorocyclopentadiene. The octachlorocyclopentene was thermally dechlorinated to hexachlorocyclopentadiene by the procedure described by Burton, Potter, and Silverman (*1*). The yield was 6.75 grams. This is 98% based on octachlorocyclopentene and 43.6% based on barium carbonate.

Aldrin. Aldrin was prepared by the reaction of hexachlorocyclopentadiene with 2,5-norbornadiene (*1*). The product was distilled at a pressure of 30 microns, giving a yield of 8.48 grams of crude aldrin. The infrared spectrum of this product indicated that it was contaminated with a small amount of isodrin, a geometrical isomer of aldrin. The presence of isodrin was detected by an absorption band at 13.58 microns which is not present in pure aldrin.

To remove the isodrin from the aldrin, the crude product was chromatographed on an alumina column which was 4 cm. in diameter and 115 cm. long. The alumina was Fisher 80- to 200-mesh adsorption grade which was activated by heating at 200° C. *n*-Hexane was used as the eluting solvent. The aldrin was eluted first, 7.3 grams of isodrin-free aldrin being collected. This is 81.0% based on hexachlorocyclopentadiene or 35.3% based on barium carbonate. The succeeding fractions contained a mixture of radioactive isodrin and aldrin. They were combined and reserved for further separation.

The aldrin at this point was almost pure. The infrared spectrum was identical with that of a pure authentic sample and the melting point was 98° C. as compared with 102.5° C. for the authentic sample. A 3.86-gram portion was recrystallized from 11 ml. of isopropyl alcohol with the use of the recrystallizing apparatus shown in Figure 4. Recovery was 3.1 grams of pure radioactive aldrin, melting point 102.5° C. This represents a 65% yield from hexachlorocyclopentadiene and a 28.3% yield from barium carbonate. The specific activity was 3.6 mc. per gram.

Dieldrin. Using the method of Burton *et al.* (*1*) as a guide, the following procedure was used to convert aldrin to dieldrin: A 3.96-gram portion of chromatographed, but not recrystallized, radioactive aldrin was placed in a 125-ml. glass-stoppered Erlenmeyer flask. To this were added 70 ml. of 0.5*M* perbenzoic acid in chloroform. The reaction mixture was allowed to stand for 17 hours at 6° C. It was then transferred to a 250-ml. separatory funnel and washed once with 50 ml. of a 10% sodium bisulfite solution, twice with 50 ml. of saturated sodium carbonate solution, and three times with 50 ml. of water. The chloroform solution was transferred to a 300-ml. flask and the solvent was removed with a rotary evaporator. The crude product was vacuum-transferred to the recrystallizing apparatus shown in Figure 4. The transfer was effected by heating the dieldrin to 180° C. at 60-micron pressure.

The product was recrystallized twice from 10 ml. of isopropyl alcohol to give a yield of 2.58 grams of pure dieldrin or 62.6% based on aldrin. The infrared spectrum was identical with that of an authentic sample of pure dieldrin and the melting point, 181° C., was the same as that of the authentic sample. The over-all yield of dieldrin based on barium carbonate was 22%. The specific activity was 1.32 mc. per mmole or 3.5 mc. per gram.

Because 79 mc. of C^{14} in 200 moles of barium carbonate were used, dieldrin with a specific activity of 4.2 mc. per gram would be expected. However, the 19% dilution of radioactive trichloroethylene with inert trichloroethylene in the preparation of octachlorocyclopentene results in a net expected activity of 3.4 mc. per gram. This is in remarkably good agreement with values obtained and indicates that no exchange of C^{14} occurred in any of the reactions involved.

Acknowledgment

The authors express their appreciation to the Agricultural Research Division, Shell Development Co., Modesto, Calif., and particularly to John C. Potter and William B. Burton of that company for valuable information and suggestions during the course of this work.

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MOLECULAR COMPLEXES OF 1,1,1-TRICHLORO-2,2-BIS (p-CHLOROPHENYL) ETHANE WITH AROMATIC DONORS

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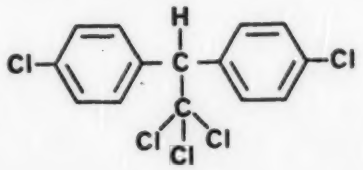
Received April 13, 1970

SUMMARY

A series of complexes between DDT acting as an electron acceptor and certain aromatic electron donors, including benzene, toluene, durene, mesitylene, pentamethylbenzene, and hexamethylbenzene, have been determined in solution using nuclear magnetic resonance spectroscopy. Variations in the proton chemical shifts between complexed and uncomplexed acceptor molecules are found to change relative to one another in the presence of excess donor. The sensitivity to small changes in the electrical environment of the magnetic nucleus of the acceptor molecule in the presence of donor compounds and the study of appropriate nuclear magnetic resonance parameters affords useful information concerning these complexes. Equilibrium constants for π-complex formation in $CHCl_3$ and CCl_4 have been determined by the Benesi-Hildebrand method or a modification of it.

The effects on the public health of certain chlorinated hydrocarbons commonly used as pesticides, such as DDT, are of increasing interest. Many workers (1-4), in pointing out current ignorance about the mechanism of action of these pesticides, have stressed the need for studies aimed at establishing, on a molecular level, the basis of activity of the pesticides and their metabolites. Their toxicity is thought to be due to effects produced on the central nervous system of both vertebrates and invertebrates. DDT's mode of action *in vivo* has



DDT (1,1,1-trichloro-2,2-bis(p-chlorophenyl) ethane)

been studied by a number of investigators. Gunther *et al.* (5) proposed that

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Van der Waal's forces were involved in the binding of nerve protein with DDT, thus precipitating its toxic effects. Mullins (6) suggested that its toxicity may be due to its ability to fit into an inter-molecular lattice and act as a plug to prevent adequate ionic diffusion. Other workers (7) explain its toxicity in terms of disruption of proper balance of K^+ ion transport in the nervous system. A more specific postulate is that DDT selectively inhibits the action of an Na^+, K^+, Mg^{2+} - adenosine triphosphatase found in the nerve ending fraction of the rat brain (8). A recent, somewhat controversial, proposal for the mode of action of DDT is the charge transfer complex (π-complex) formation, a hypothesis of Matsumura and O'Brien (9). They have suggested that DDT may act by forming a charge transfer complex with protein components of the nerve axon, i.e., the formation of molecular complexes between electron donor (D) and electron acceptor (A) molecules by partial transfer of an electron from the donor to the acceptor. Although no causal relationship between the formation of charge transfer complexes and symptoms of poisoning has been established, the questions arising from this proposal prompted a further investigation of this assumption and collection of some quantitative data for π-complex formation of DDT with more common electron donors.

Certain molecular species are known to form π-complexes with aromatic and olefinic hydrocarbons (10, 11). The structure and chemistry of these complexes have been extensively investigated (12, 13), although only a few of the known complexes have been studied in detail. Visible, ultraviolet, and infrared spectroscopic techniques, and more recently, nuclear magnetic resonance (NMR) (14), have been useful in providing data about the structure of the complexes.

The method for obtaining equilibria data for π-complexes of this type, described by Benesi and Hildebrand (15), takes advantage of the fact that most π-complexes have a new absorption band in the visible or ultraviolet region of the spectrum which is characteristic of the complex itself. From plots of optical density versus donor concentration, the equilibrium constants and molar absorbancy index may be calculated.

A modification of the Benesi-Hildebrand equation (15) for determining equilibrium constants of this type has been derived for NMR applications. By considering the chemical shift of protons on molecules undergoing rapid exchange between complexed and uncomplexed states and by following treatments used in NMR studies of hydrogen bonding equilibria (16-18) the equation shown below may be derived.

$$\frac{1}{\Delta^{A}_{OBS}} = \frac{1}{K\,\Delta^{A}_{AD}\,c_D} + \frac{1}{\Delta^{A}_{AD}}$$

Δ^{A}_{OBS} is the observed shift of acceptor protons in the complexing medium; Δ^{A}_{AD} is the shift of acceptor protons in the pure complex; c_D is the concentration of donor in moles per kilogram of solvent; K is the equilibrium constant. This equation requires that $c_D >> c_A$. It also assumes that solutions are ideal or that the quotient $\frac{(\gamma AD)}{(\gamma A)(\gamma D)}$ remains constant over the range of solutions studied.

The utility of NMR in the study of π-complex formation has been demonstrated (14, 19, 20) by the close agreement between results obtained by the NMR method and those obtained by other spectroscopic techniques.

The observed chemical shifts of the benzylic proton of DDT upon addition of some aromatic donors in CCl_4 and $CHCl_3$ solutions are reported in this paper. These shifts are interpreted as resulting from charge transfer complex formation of DDT with these compounds. Some data concerning the structure of the complex itself and the magnitudes of the equilibrium constants of these complexes are also discussed.

EXPERIMENTAL

Materials: The DDT used was an analytical standard (98.9% purity) obtained from Aldrich Chemical Co. and used without further purification. All liquids were spectroquality reagents. Tetra-, penta-, and hexamethylbenzene were purified in the usual manner (21).

Procedure: A standard stock solution of DDT in both CCl_4 and $CHCl_3$ was prepared with the concentration just sufficient to observe an NMR signal for the benzylic

proton. A known amount of donor was weighed in a tared flask, 5 ml of DDT stock solution was added, and the flask was reweighed.

Instrumentation: The NMR spectra were recorded on a Varian Associates HA-100 high resolution spectrometer operating at 100 MHz. The line positions of the proton resonance were measured with a Hewlett Packard Model 5512 A electronic counter, which displays the pen position in cps relative to an internal tetramethylsilane (TMS) reference. Shifts were measured to an estimated accuracy of 0.25 Hz. All shifts in the signal caused by addition of electron donor to the system were to higher field values. Measurements were made at 36.2±1.0°, the ambient temperature in the vicinity of the probe.

Equilibrium constants were calculated from the NMR data using a least squares regression program and a Wang Model 300 series electronic calculator.

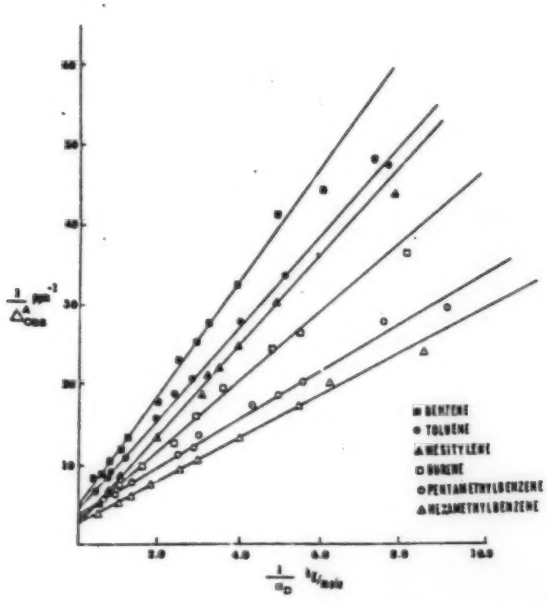

Fig. 1. Plots of $1/\Delta^{A}_{OBS}$ vs $1/m_D$ for the NMR shifts of the benzylic proton of DDT with aromatic donors in CCl_4 solution.

RESULTS AND DISCUSSION

Figures 1 and 2 show plots of the reciprocal of the observed shifts of acceptor protons versus the reciprocal of the concentration of donor molecules in CCl_4 and $CHCl_3$ solutions respectively. Good straight lines were obtained in all cases. The increased deviation at high values of $1/m_D$ is to be expected since this corresponds to low molalities of donor and to a breakdown of the approximation that $c_D >> c_A$. Tables 1 and 2 summarize the data for the range of donor concentrations, the observed shifts, the calculated values of equilibrium constants, and the chemical shifts of acceptor protons in the pure complex. The validity of the data in the tables is demonstrated by a plot of Δ_{OBS} vs m_D for the DDT-benzene system, Fig. 3. The conclusion can be drawn that K's determined from NMR measurements are valid as long as a plot of Δ_{OBS} vs the appropriate concentration gives a curved line (14).

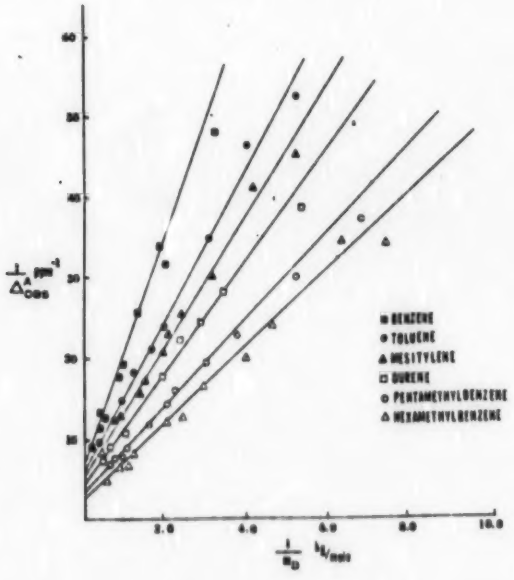

Fig. 2. Plots of $1/\Delta^{A}_{OBS}$ vs $1/m_D$ for the NMR shifts of the benzylic proton of DDT with aromatic donors in $CHCl_3$ solution.

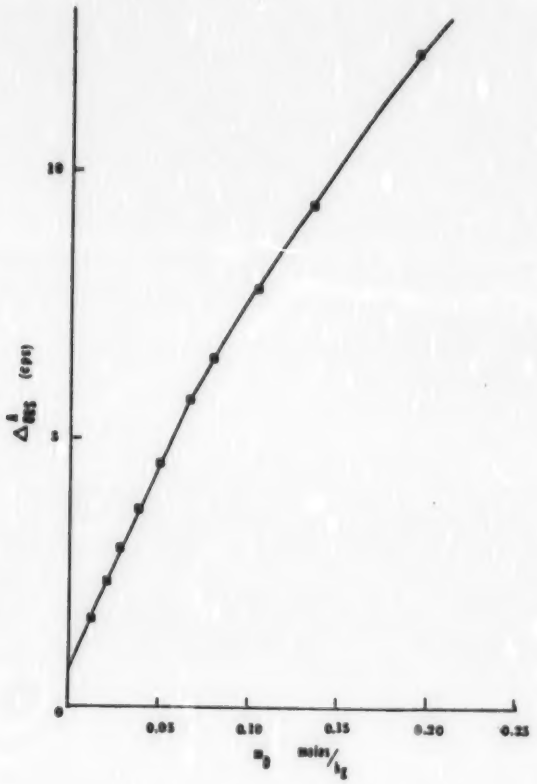

Fig. 3. A plot of Δ^{A}_{OBS} vs m_D for the NMR shifts of the benzylic proton of DDT with aromatic donors in CCl_4 solution.

Due to solubility the concentration range studied varied with each donor. As the concentration of donor was increased, an increase in the maximum observed shift was detected. Also, as the ionization potential of the donor increased, the observed shifts became smaller. Measurements to determine K were made by the acceptor methine proton resonance, and contributions to the observed values of the maximum observed shifts due to self-complexing are considered to be small. Since the complex can exist only in solution in equilibrium with donor and acceptor, the possibility of complex formation between the complex and another molecule (or another donor-acceptor species) was not determined.

The variations of K (Tables 1 and 2) in CCl_4 and $CHCl_3$ cannot be wholly

Table 1. A Summary of the Properties of π-Complex Formation of DDT with Aromatic Molecules in Carbon Tetrachloride Solution

Donor	Range of Donor Concentration (moles/kg Solvent)	Δ_{max} Observed (cps)	Δ^{A}_{AD} (cps)	K (kg/mole)
Benzene	0.05 - 3.5	17.0	24.5	0.034
Toluene	0.05 - 3.0	20.0	23.4	0.040
Mesitylene	0.05 - 2.0	17.0	35.8	0.065
Durene	0.10 - 0.80	28.0	60.2	0.135
Pentamethylbenzene	0.10 - 0.80	29.0	41.2	0.112
Hexamethylbenzene	0.10 - 0.50	20.0	52.6	0.189

Table 2. A Summary of the Properties of π-Complex Formation of DDT with Aromatic Molecules in Chloroform Solution

Donor	Range of Donor Concentration (moles/kg Solvent)	Δ_{max} Observed (cps)	Δ^{A}_{AD} (cps)	K (kg/mole)
Benzene	0.05 - 3.5	18	18.2	0.013
Toluene	0.05 - 3.0	18	18.5	0.019
Mesitylene	0.05 - 2.0	25	22.4	0.027
Durene	0.10 - 0.80	17	27.5	0.034
Pentamethylbenzene	0.10 - 0.80	28	26.9	0.050
Hexamethylbenzene	0.10 - 0.50	18	33.0	0.060

attributed to the dielectric constant, refractive index, or other physical properties of the solvent. Probably the most important cause for the discrepancies in the values of K in the two solvents is the competition of solvent for donor molecules. This effect is greater for $CHCl_3$ than for CCl_4, and may account for the smaller values obtained for K in $CHCl_3$ (22).

We observed no definite trends in the values of the shift of the acceptor protons in the pure complex with the structure of the donor in comparing our results with values obtained by previous workers (14, 20). The same magnitude

of differences in the shift of the protons of pure complex is obtained for the same complex in CCl_4 and $CHCl_3$. It must be noted that absolute magnitude of the observed shift of acceptor protons is a function only of the structure of the actual complex and should be independent of the concentration scale.

Several observations may be used to support the assumption that these observed shifts for complex formation of DDT with the aromatic donors are due to molecular complexing. The equilibrium constants are in the right order, i.e., as the ionization potential becomes lower, the equilibrium constant increases. When DDT was replaced with toluene no shift of the toluene protons was observed. These observations strongly suggest that the observed shifts of the benzylic proton of DDT are due not to some kind of general solvent effect but to molecular complexing.

In determining equilibrium data, the major advantage of NMR over other spectroscopic techniques is that is is possible to study complexes that have no separate charge transfer band in the visible or ultraviolet spectrum, or where the charge transfer band is obscured by the spectrum of one of the other components. Also, it is easier to measure line position than an intensity (23). The system investigated in this work was ideal for NMR study in several ways: (1) The benzylic proton of DDT affords a single sharp resonance line at approximately 495 Hz. (2) This absorption peak does not overlap with the absorption peaks of the donor molecules studied, making it easy to measure its position. (3) We were able to make the donor concentration large with respect to the acceptor molecule, DDT. (4) The absorption peak observed did not overlap with the absorption peaks of the two solvents used in this study. Since it is difficult to find an experimental system in which the same or similar conditions are true, care must be taken in choosing the model system for study of complex formation by this technique.

This work has demonstrated the existence of charge transfer complexes of DDT in nonaqueous solution. We are engaged in a variable temperature study of this system to obtain some data for the bond energies and other physical con-

stants for these complexes. Also, we are searching for a suitable solvent with which to extend the use of this method to further investigate the complex formation of DDT with suitable biological substrates *in vitro* as a possible approach to determining the mode of action of this compound.

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